Polyphenols and Rosmarinic acid Contents, Antioxidant and Anti-Inflammatory Activities of Different Solvent Fractions from Nga-Mon (*Perilla frutescens*) Leaf

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Abstract: Perilla is a rich source of polyphenols, which exhibits antioxidant, anti-inflammatory activities, and a variety of biological effects. The effect of differential solvents on the polyphenols, flavonoids, rosmarinic acid (RA), anti-inflammatory and antioxidant activities of perilla leaf require investigation. In this study, perilla leaf was extracted with 70% ethanol and sequentially fractionated according to the solvent’s polarity with hexane, dichloromethane, ethyl acetate, and water. Samples were subjected to the bioactive compound measurements. The antioxidant and anti-inflammatory nature of perilla was analyzed based on the scavenging effects on DPPH, ABTS⁺, O₂⁻ and nitric oxide (NO), as well as FRAP assay, and determination of the inhibition effects on NO, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) production in the cell-based study. The results indicate that among all different solvents used for sequential fractionation, ethyl acetate (EIOAc) was most effective in the separation of anti-oxidative and anti-inflammatory compounds in the perilla leaf extract. These properties can partly be due to the presence of polyphenolics, flavonoids, and also RA. It can be demonstrated here that, the perilla leaf EIOAc fraction could be used as a natural active pharmaceutical ingredient for dietary supplements and nutraceuticals.

Keywords: *Perilla frutescens*, Polyphenols, Rosmarinic acid, Antioxidant, Anti-inflammation.

INTRODUCTION

Overproduction of free radicals can cause oxidative stress and oxidative damage to the biological macromolecules, including protein, lipid, and nucleic acid, which are associated with metabolic diseases [1]. Furthermore, free radicals such as nitric oxide (NO) synthesized by three isoforms of the enzyme nitric oxide synthase (NOS), play an important role in the inflammatory process and also induce cyclooxygenase (COX) production in the inflammatory process, and increase the production of interleukin-1 (IL-1) and tumor necrosis factor (TNF). The toxicity of NO increases when it reacts with the superoxide radical (O₂⁻), forming the highly reactive peroxynitrite (ONOO⁻) [2].

Many researchers indicated that plant-based antioxidants possess great therapeutic agents against oxidative stress closely associated with chronic inflammation. However, in the present, synthetic antioxidants have been used to scavenge the free radical, but due to their side effects leading to carcinogenicity, search for effective and natural antioxidants has become necessary [3].

*Perilla Frutescens* (perilla or Nga-mon in Thai) is a herb that belongs to the mint family, traditionally grown in East Asia, mainly Northern Thailand [4]. It is used in local wisdom for various purposes including medicine and cosmetics, edible oil, as a herb in salads, sushi, soups, and as a spice, as well as a food decoration or a colorant. The perilla leaves have a sweet taste, containing about 3.1% protein, 0.8% fat, 4.1% carbohydrate, and 1.1% ash. The young leaves are used as a spice, older leaves used as a food decoration or flavoring and wound healing. In particular, perilla leaves exhibit several health benefits due to a high content of polyphenols, flavonoids, including rosmarinic acid [5]. Recently, it was shown that Thai perilla leaf inhibited the invasion and migration of human breast cancer [6].

Rosmarinic acid (RA) is a polyphenolic compound (ester of caffeic acid and 3, 4-dihydroxyphenyllactic acid) that is commonly found in many plant species including rosemary, selfheal, lemon balm, sage, mint, and basil [7]. RA has been reported as a potent
antioxidant and anti-inflammatory agent. Moreover, RA also has been shown the ability to reduce liver injury by inducing D-galactosamine and lipopolysaccharides, through the scavenging of superoxide molecules and the inhibition of COX-2 production [8]. RA has many complex actions on anti-inflammatory effect; it has shown the activation of reciprocal inhibition both in vivo and in vitro [9]. RA inhibits cytokine release from activated T cells and also limits the production of pro-inflammatory mediators [10].

The extract yield of plants depends on the capacity and polarity index (PI) of the solvents. The PI is a measure of the ability of any solvent to dissolve solutes with opposite charges. The PI of a solvent increases with its polarity and dielectric constant [11, 12]. Previous studies have indicated that the PI influences polyphenol extraction and bioactivities [13, 14]. Therefore, the PI can be used to identify suitable solvents for the extraction of polyphenol compounds from perilla leaf. However, other factors that are involved in extraction capacities such as temperature, time, pH, and chemical structure of the components, are also important [15, 16]. Until now, the effect of solvent polarity and extraction capacity of polyphenols flavonoids, RA, as well as the antioxidant and anti-inflammatory activity of perilla have not been reported. Thus, this research aims to understand the changes in phytochemical content, cytotoxicity, antioxidant, and anti-inflammatory activities of perilla leaf according to the different polarities of solvents used in the extraction and partition method.

MATERIALS AND METHODS

Collection of Plants and Preparation of Extracts

Perilla leaves were collected from Wiang-Sa district, Nan province, Thailand. The voucher specimen code is QSBB-K2, prepared by Dr.Komsak Pintha and Dr.Payungsak Tantipaiboonwong, and certified by the Queen Sirikit Botanic Garden Herbarium, Chiang Mai, Thailand. Dried perilla leaves were extracted with 70% ethanol (EtOH) to obtain the EtOH crude powder which was then sequentially partitioned with hexane (Hex), dichloromethane (DCM), ethyl acetate (EtOAc), and residue aqueous phase is water (H₂O). The percent yield of extracts and fractions were calculated as % w/w dry base. Each fraction was stored at 20°C and suspended in dimethyl sulfoxide (DMSO) before use.

Total Phenolic Content (TPC)

TPC was determined using the Folin–Ciocalteu method of Hossain et al. [17]. Briefly, 20 µL of the sample was mixed with 100 µL of 10% Folin–Ciocalteu reagent and 80 µL of 7.5% Na₂CO₃. After 30 mins at room temperature, the absorbance at 765 nm was measured, and TPC was estimated using a standard curve of gallic acid. TPC was expressed as milligram gallic acid equivalent per 1 g fraction (mg GAE/g fraction).

Total Flavonoid Content (TFC)

TFC was examined using the aluminum chloride colorimetric method with slight modification [18]. Initially, 25 µL of the fraction and 125 µL deionized water were mixed with 7.5 µL of 5% NaNO₂ solution and incubated at room temperature for 6 min. Then, 15 µL of 10% AlCl₃ was added and incubated for another 6 min. Color development was performed by adding 50 µL of 1 M NaOH. The final volume of the reaction mixture was adjusted to 250 µL using deionized water. The absorbance was measured at 510 nm. TFC was calculated using a standard curve of catechin and expressed as milligram catechin equivalent per 1 g fraction (mg CE/g fraction).

Measurement of RA

RA was determined by reversed-phase HPLC using the Agilent 1200 equipped with the multi-wavelength and fluorescence detectors. The assay was carried out using a Symmetry Shield RP18 column (4.6 mm × 250 mm, 5 µm particle diameters, Waters Co., Ltd.), and 30% acetonitrile in 0.1% acetic acid and H₂O was used as a mobile phase with a flow rate 1.0 mL/min. The peaks were detected using a UV detector at 330 nm. The rosmarinic acid content was rechecked with a fluorescence detector with excitation of wavelength at 330 nm and emission of wavelength at 400 nm. All samples were measured in triplicates.

In Vitro Antioxidant Activity-Scavenging Effect

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

The DPPH free radical scavenging assay was performed as previously described [19]. Various concentrations of each sample (20 µL) were mixed with 180 µL of freshly prepared DPPH methanolic solution and kept in the dark for 30 mins before measuring with an AccuReader microplate reader (Metertech Taiwan) at 540 nm. The standard curve was of ascorbic acid. Results were expressed as 50% DPPH decolorization (IC₅₀).
2,2′-Azino-Bis-3-Ethylbenzthiazoline-6-Sulfonic Acid (ABTS) Radical Scavenging Assay

The ABTS free radical-scavenging assay was also performed using the method explained above [20] but with slight modifications. The ABTS solution was dissolved in potassium persulfate and kept in the dark for 12–14 h. Before use, this solution was diluted with distilled water to get an absorbance at 734 nm of approximately 0.70. The various concentrations of each fraction (10 µL) were mixed with 990 µL of working diluted ABTS and incubated for 6 min in the dark. The absorbance was measured at 734 nm. The standard curve was of ascorbic acid and Trolox. Results showed 50% ABTS decolorization (IC_{50}).

Superoxide Anion Radical Scavenging Assay

The scavenging effects on superoxide anion of samples were assayed according to the method of Saenjum et al. [21]. This method is based on the power of the samples to inhibit formazan formation in a phenazine methosulfate (PMS) - β-nicotinamide adenine dinucleotide (NADH) system. Superoxide anion radicals were generated in an NADH-PMS system through the oxidation of NADH and then analyzed by the reduction of nitroblue tetrazolium (NBT). The reaction mixture was made in PBS buffer (pH 7.4), which contained 78 µM of NADH, 25 µM of NBT and 45 µM of EDTA, and combined with different concentrations of the tested samples or positive controls (L-ascorbic acid and RA). PMS was added to initiate the reaction, and after 5 min of incubation in the dark, the absorbance was measured at 560 nm. All samples were tested in triplicate. The decreased absorbance indicated increased superoxide anion scavenging activity.

Nitric Oxide Radical Scavenging Assay

In vitro NO-scavenging activity is analyzed using Griess reaction. The reaction mixture is composed of 6.25 M sodium nitroprusside in PBS buffer and positive control (curcumin and RA) or tested samples. The reaction mixtures were incubated at 37°C for 150 min. Then, the reaction mixture was transferred to a 96-well plate. The Griess reagent, a mixture of naphthylethylene diamine and sulphanilamide was added and incubated at room temperature for 5 min. The absorbance was measured at 540 nm. All samples were tested in triplicate.

Ferric Reducing/Antioxidant Power (FRAP) Assay

40 µL of each extract was mixed with 3 mL of FRAP reagent, and the reaction mixture was incubated at 37°C for 4 min before it was measured at 593 nm using a spectrophotometer. The blank solution consisted of 40 µL distilled water in 3 mL FRAP reagent and was incubated at 37°C for 1 h. The standard solutions consisted of FeSO_{4}·7H_{2}O in different concentrations. The results were expressed as mg Fe (II) per 1 g fraction (mg Fe(II)/g fraction).

Anti-Inflammatory Activity -Cell-Based Assay

Cell Viability Assay

RAW 264.7 mouse macrophage cells viability of all samples in the concentrations of 10, 25, 50, 100, and 200 µg/mL was measured using cell proliferation reagent WST-1 (Roche, Germany).

Inhibition of NO, iNOS, and COX-2 Production

Briefly, RAW 264.7 cells were cultured with DMEM in 24-wells plate for 24 h. Then, cells were replaced with a new medium containing various concentrations of tested samples (10 – 100 µg/mL: non-toxic dose) and incubated for 12 h. After that, lipopolysaccharide (LPS) and interferon-γ (IFN-γ) was added and incubated for 48 h, the culture supernatants were collected to measure for NO production using Griess reaction, and the cell lysates were measured for iNOS and COX-2 using immunoassay kit. The results were represented as 50% inhibitory concentration values (IC_{50}). Curcumin and RA were used as a positive control [22].

Statistical Analysis

Data are shown as the mean ± standard deviation of three independent experiments. The statistical analysis was determined using one-way analysis of variance. Significant differences at the level of p < 0.05 were determined by Tukey’s multiple comparison test, and data correlation was obtained by Pearson correlation test, using IBM SPSS Statistics 22.

RESULTS

Yield and Physical Properties

The percent yield of ethanolic crude extract (PE) was 13.4% w/w. The crude extract was sequentially partitioned with Hex, DCM, EtOAc, and H_{2}O fractions. The results showed that the lower yield was noted in H_{2}O fractions (Table 1). Each fraction produced different yields, various colors, and appearance. This may be due to the presence of phytochemicals in the fractions, the solvent used, and it is PI [23].
TPC, TFC and RA Content

As illustrated in Table 2, the highest TPC was detected in the EtOAc fraction and the lowest in DCM fraction. Inconsistent with the TPC results, the TFC of EtOAc fraction was exceptionally high, and the declining level was sequenced by EtOH extract, water, hexane, and DCM fraction. The presence of RA in perilla leaf fractions were studied by reversed-phase HPLC analysis. The chromatogram is shown in Figure 1. The EtOAc and DCM fraction contained the highest and lowest amount of RA. As evident from the table, the amount of RA from the EtOAc fraction was approximately 100-fold higher than that of the DCM fraction. Moreover, the results of RA content in all analyzed samples were correlated to TPC and TFC content with $r^2$ 0.978 and 0.994 ($p<0.01$), respectively.

**In Vitro Antioxidant Activity**

The ability of ethanolic extract and their fractions in various solvents to scavenge free radicals were analyzed using DPPH and ABTS assay, as well as NADH-PMS system assay and Griess reaction assay. The calculated IC$_{50}$ values denote the concentrations of the fraction required to decrease the scavenging activity by 50%. Moreover, the results are shown in Table 3. The radical scavenging showed that the EtOAc fraction possessed the highest antioxidant activity.

Table 1: Percent Yield and Physical Properties of Perilla Fractions

<table>
<thead>
<tr>
<th>Fractions*</th>
<th>Solvents</th>
<th>Polarity index (PI)</th>
<th>% Yield</th>
<th>Physical Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
<td>Ethanol (EtOH)</td>
<td>5.2</td>
<td>13.4</td>
<td>Green-brownish powder</td>
</tr>
<tr>
<td>PHF</td>
<td>Hexane (Hex)</td>
<td>0</td>
<td>42.5</td>
<td>Dark-brownish sticky</td>
</tr>
<tr>
<td>PDF</td>
<td>Dichloromethane (DCM)</td>
<td>3.7</td>
<td>14.5</td>
<td>Dark green-brownish sticky</td>
</tr>
<tr>
<td>PEF</td>
<td>Ethyl acetate (EtOAc)</td>
<td>4.4</td>
<td>12.2</td>
<td>Yellow-brownish sticky</td>
</tr>
<tr>
<td>PWF</td>
<td>Water ($H_2O$)</td>
<td>9</td>
<td>30.8</td>
<td>Brown powder</td>
</tr>
</tbody>
</table>

*Abbreviation.

Table 2: Total Phenolic, Flavonoid, and RA Content of Perilla Fractions

<table>
<thead>
<tr>
<th>Fractions</th>
<th>TPC mg GAE/g fraction</th>
<th>TFC mg CE/g fraction</th>
<th>RA mg /g fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
<td>138.3 ± 6.6$^b$</td>
<td>193.5 ± 12.1$^c$</td>
<td>69.1 ± 1.8$^d$</td>
</tr>
<tr>
<td>PHF</td>
<td>28.7 ± 1.8$^a$</td>
<td>39.7 ± 5.2$^a$</td>
<td>6.6 ± 0.7$^b$</td>
</tr>
<tr>
<td>PDF</td>
<td>28.0 ± 2.2$^a$</td>
<td>25.5 ± 6.3$^a$</td>
<td>2.9 ± 0.6$^a$</td>
</tr>
<tr>
<td>PEF</td>
<td>344.0 ± 21.9$^b$</td>
<td>575.5 ± 18.1$^d$</td>
<td>303.3 ± 6.4$^e$</td>
</tr>
<tr>
<td>PWF</td>
<td>128.1 ± 8.1$^c$</td>
<td>150.8 ± 14.3$^c$</td>
<td>45.2 ± 0.9$^c$</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± standard deviation (SD; n = 3). Different letters indicate a significant difference ($p < 0.05$).

Figure 1: HPLC chromatograms of a mixed standard solution containing RA, rutin, and quercetin (A) and EtOAc fraction (B).
activity. The DCM fraction exhibited less scavenging activity. Surprisingly, nitric oxide radical scavenging activity in EtOAc fraction was almost equal to rosmarinic acid, which was used as a positive control. Beside scavenging assay, antioxidant activity was also measured by ferric reducing/antioxidant power assay; the highest and lowest antioxidant capacity was also observed in the EtOAc and DCM fractions, respectively.

**Cell-Based Study of Anti-Inflammatory Activity**

To evaluate the effect of the perilla leaf extracts on the LPS and IFN-γ-stimulated the production of NO, iNOS, and COX-2, Griess reaction assay and ELISA were measured. As displayed in Table 4, amongst the fractions, the most active response was seen in the EtOAc fraction in all inflammatory proteins. The weak inhibition was found in the DCM fraction.

Previous literature demonstrated that the antioxidant and anti-inflammatory activity of various fruits and vegetables are significantly correlated to their content of the polyphenolics. The Pearson correlation test illustrated that TPC, TFC, and RA level were correlated with antioxidant scavenging activities and anti-inflammatory activity. Interestingly, there was a remarkable correlation between the relatively high amount of TPC, TFC, RA and the reducing power activity (FRAP) with $r^2$ 0.992, 0.997, and 0.994; $p<0.01$, respectively (Table 5). According to this rationale, the EtOAc fraction had a high content of polyphenolic compounds, which are linked to antioxidant and anti-inflammatory activity.

**DISCUSSION**

Perilla or Nga-mon in Thai is commonly known to exhibit beneficial health activities owing to the appearance of phenolic and flavonoid compounds such as apigenin, luteolin, caffeic acid, and RA, including vitamins and essential unsaturated fatty acids [24]. Nowadays, literature has evaluated that antioxidant

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**Table 3: Antioxidant Activities of Perilla Leaf Fractions**

<table>
<thead>
<tr>
<th>Fractions &amp; standards</th>
<th>Reducing power assay (mg Fe (II)/g fraction)</th>
<th>Radical scavenging assay IC$_{50}$ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FRAP</td>
<td>DPPH</td>
</tr>
<tr>
<td>PE</td>
<td>1,326.6 ± 67.4$^a$</td>
<td>23.0 ± 3.2$^a$</td>
</tr>
<tr>
<td>PHF</td>
<td>279.1 ± 12.2$^a$</td>
<td>110.6 ± 5.9$^a$</td>
</tr>
<tr>
<td>PDF</td>
<td>213.0 ± 12.7$^a$</td>
<td>157.6 ± 7.7$^a$</td>
</tr>
<tr>
<td>PEF</td>
<td>4,759.1 ± 183.6$^b$</td>
<td>9.1 ± 0.5$^b$</td>
</tr>
<tr>
<td>PDF</td>
<td>1,328.9 ± 16.2$^b$</td>
<td>99.4 ± 6.4$^b$</td>
</tr>
<tr>
<td>L-ascorbic acid</td>
<td>13.6 ± 1.3$^b$</td>
<td>2.1 ± 0.0$^b$</td>
</tr>
<tr>
<td>RA</td>
<td>13.1 ± 0.6$^b$</td>
<td>9.6 ± 0.6$^b$</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± standard deviation (SD; n = 3). Different letters indicate a significant difference ($p < 0.05$).

**Table 4: Inhibition of NO, iNOS, and COX-2 Production**

<table>
<thead>
<tr>
<th>Fractions &amp; standards</th>
<th>IC$_{50}$ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO</td>
</tr>
<tr>
<td>PE</td>
<td>26.4 ± 1.0$^d$</td>
</tr>
<tr>
<td>PHF</td>
<td>33.3 ± 1.4$^a$</td>
</tr>
<tr>
<td>PDF</td>
<td>44.3 ± 1.5$^d$</td>
</tr>
<tr>
<td>PEF</td>
<td>17.9 ± 0.7$^a$</td>
</tr>
<tr>
<td>PWF</td>
<td>24.1 ± 1.1$^d$</td>
</tr>
<tr>
<td>RA</td>
<td>13.2 ± 0.6$^b$</td>
</tr>
<tr>
<td>Curcumin</td>
<td>7.5 ± 0.7$^a$</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± standard deviation (SD; n = 3). Different letters in each group (leaves and seed meal) indicate a significant difference ($p < 0.05$).
and anti-inflammatory activities of perilla are due to the various bioactive compounds. Additionally, the extraction efficiency of phytochemicals strongly depends on the fractionation conditions, solvent polarity, temperature, compound preparation, structure, and size of the molecule [15, 18].

In current studies, it has been observed that the lower yield of extractable compounds was observed in EtOAc and DCM fractions in comparison to the Hex and H2O fractions. Also, different colors and appearance of fractions were noticed in each sample. The difference may be due to the presence of different ingredients such as polyphenols, chlorophyll, resin, tannin, nutrients, and minerals in the fractions [25]. The pattern and correlation of TPC, TFC, and RA distribution in perilla extracts investigated in our research were in agreement with the results of Korean and Chinese investigators [26, 27], which recently also demonstrated that the EtOAc fraction of perilla leaves had the highest content of polyphenols, flavonoids, and RA. Perilla parts also contain other polyphenolics such as luteolin, apigenin, caffeic acid, and their glucosides, as well as ferulic acid, vanillic acid, chlorogenic acid, and 4’,5,7-trimethoxyflavone [28].

The polarity of a solvent is a primary determinant of the number of polyphenols extracted compounds. In this study, EtOAc (PI = 4.4) was found to be the most effective solvent for the extraction of polyphenolics, flavonoids, and RA from perilla leaf. Normally, polyphenolics, flavonoids, and RA contain carboxyl and hydroxyl groups, which easily form hydrogen bonds with polar solvents [29, 30]; therefore, H2O, EtOH, and EtOAc, which have higher PI than the Hex and DCM fractions [31] can separate more polyphenol compounds then Hex and DCM. Thus, we can conclude that the solubilization of polyphenols in the EtOAc fraction is related to the PI of the solvent. Typically, antioxidants are compounds that can delay or inhibit the oxidation of lipids and other molecules, and act by one or more of the following mechanisms; reducing activity, free radical scavenging, possible complexation of pro-oxidant metals, and quenching of singlet oxygen [32]. As seen in the present study, the various scavenging capacities by different assays may be due to the principle and mechanism [33-35]. Our results from the different antioxidant activities demonstrated the similar trend that the EtOAc and DCM fractions showed, represented the highest and the lowest free radical scavenging activity and were consistent with that reported in the literature [26-28, 36]. Moreover, a cell-based anti-inflammatory activity found that perilla ethyl-acetate fraction can also inhibit LPS and IFN-γ-stimulated the production of NO, iNOS, and COX-2 in RAW 264.7 mouse macrophage cells. In our results, the Pearson correlation test illustrated that total phenolic, total flavonoid, and RA content was correlated with antioxidant scavenging activities and with anti-inflammatory activity.

According to this rationale, by sequential fractionation using different solvents, EtOAc, which is semi-polar (PI = 4.4), was found to be the most effective solvent for the extraction of total polyphenols and the isolation of RA. Our data confirmed that the PI of solvents affects the number of phenolic compounds extracted. It is the first report comparing antioxidant and anti-inflammatory activity and polyphenols in solvent extracts of perilla leaves. The EtOAc extract of perilla leaves could thus be a valuable source of RA to be used as a natural active pharmaceutical ingredient in dietary supplements and nutraceuticals.

| Table 5: Pearson Correlation Coefficients (r²) between Assay for Perilla Leaf Fractions |
|-----------------|-----------------|-----------------|-----------------|
|                  | TPC             | TFC             | RA              |
| Reducing power activity | FRAP            |                 |                 |
| mgFe (II) /g fraction |                 |                 |                 |
| Scavenging activity (IC₁₀₀) | DPPH -0.829     | -0.818          | -0.773          |
|                   | ABTS -0.797     | -0.735          | -0.659          |
|                   | O₂ -0.69        | -0.646          | -0.577          |
|                   | NO -0.815       | -0.773          | -0.708          |
| Pro-inflammatory protein | NO -0.844      | -0.807          | -0.751          |
| inhibition (IC₁₀₀) | iNOS -0.83      | -0.842          | -0.819          |
|                   | COX-2 -0.647    | -0.638          | -0.595          |

**Correlation is significant at the 0.01 level (2-tailed).
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REFERENCES


[26] Jun Pi, Kim BT, Song GS, Kim YS. Structural characterization of phenolic antioxidants from purple perilla


