Italian Journal of Food Science, 2025; 37 (1): 78–93

Effect of sodium carbonate on nutritional composition and antioxidant activities of Indonesian *Mesona* Blume extract

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Academic Editor: Prof. Valeria Sileoni – Universitas Mercatorum, Italy

Received: 19 July 2024; Accepted: 10 October 2024; Published: 1 January 2025

ORIGINAL ARTICLE

Abstract

Mesona plant has long been used as a traditional herbal medicine to treat hyperglycemia, hypertension, hyperlipidemia, hepatic and colon disorders, and inflammation. China is the main producer of dried *Mesona*, but Indonesian *Mesona* is also popular, particularly in Thailand because of inexpensiveness and quality consistency. The nutritive values, total phenolic content, total flavonoid content, and antioxidant activities of the Indonesian *Mesona* plant aqueous extracts from both with and without sodium carbonate (control) were determined. After separation, the supernatant and precipitated extracts were analyzed for antioxidant activities, such as 2,2′-azi no-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), *2,2-diphenyl-1-picrylhydrazyl* (DPPH), and *ferric reducing antioxidant power* (FRAP). Results showed that the extracts from sample-added sodium carbonate yielded more crude protein, crude fat, and crude carbohydrate, compared to control extracts. Total phenolic contents and total flavonoid contents of extract supernatants with and without sodium carbonate were higher than the precipitated extracts with and without treatment. Supernatant extract with and without sodium carbonate showed higher values of all tested antioxidant activities based on ABTS, DPPH, and FRAP, compared to the precipitate extracts. The IC_{50} values of ABTS DPPH, and FRAP found in the supernatants with and without sodium carbonate were 0.18±0.13, 0.27±0.17, and 0.13±0.02 mg/mL and 0.25±0.02, 0.14±0.06, and 0.20±0.09 mg/mL, respectively, while the values of precipitates were 0.47±0.06, 0.61±0.02, and 0.34±0.01 mg/mL and 0.73±0.03, 0.29±0.08, and 0.32±0.04 mg/mL, respcetively. Plant extraction with addition of sodium carbonate revealed good nutritional value, total polyphenolic content, total flavonoid content, and antioxidant activities. Adding sodium carbonate improved the extraction process, with a higher yield, several bioactive compounds, and biological activities.

Keywords: antioxidant; aqueous extraction; *Mesona* Blume; nutrition; sodium carbonate

Introduction

Modern consumers prefer to eat natural food with high nutritive value that contains phytochemicals, dietary fibers, natural colorants, phenolics, minerals and vitamins, with low modified food additives and lipids (El-Samahy *et al.,* 2007). Many edible plants contain phytochemicals (Auddy *et al.,* 2003). *Mesona* Blume is an economically significant agricultural plant (Tang *et al.,* 2021) belonging to the *Lamiaceae* family and mainly distributed in China, Taiwan-China (Huang *et al.,* 2016), and in Southeast Asia, such as Malaysia, the Philippines, Indonesia, and Vietnam (Huang *et al.,* 2018; Tang *et al.,* 2022; Wang and Qin, 2014). This herb is widely consumed

 ISSN 1120-1770 online, DOI 10.15586/ijfs.v37i1.2713

as a functional beverage and soft-sticky dessert as well as a traditional medicine in China, Vietnam, and Indonesia (Huang *et al.*, 2016; Le *et al.*, 2018; Rahmah *et al.,* 2022) to treat hyperglycemia, hypertension, hyperlipidemia, hepatic disorder, colon disorder, and inflammation (Chau and Wu, 2006; Feng *et al.,* 2008; Huang and Yen, 2002; Huang *et al.,* 2021; Le *et al.*, 2018; Lin *et al.,* 2018; Yang *et al.,* 2008). *Mesona* plants from China and Taiwan-China have demonstrated bioactive potential from polysaccharide components, such as neutralizing free radical activities (Li *et al.,* 2021; Seah *et al.,* 2024), hypoglycemic and hypolipidemic activities (Li *et al.,* 2010; Xiao *et al.,* 2022), antiproliferative activities (Huang *et al.,* 2021), hepatocellular carcinoma (HepG2) cell growth inhibitory effect (Le *et al.*, 2018), anti-dyslipidemia effect (Handayani *et al.,* 2017), renal protective activity (Yang *et al.*, 2008), antihypertensive properties (Yeh *et al.*, 2009), DNA damage protection (Yen and Hung, 2000), anti-inflammatory effects (Huang *et al.,* 2012), antimutagenic effects (Yen *et al.,* 2001), and antibiosis effects (Liu and Feng, 2008). However, scientific information on *Mesona* (black cincau) from Indonesia is limited. Inexpensive and high-quality Indonesian *Mesona* is now flooding Thai markets (Seah *et al.,* 2024). Owing to medicinal, nutraceutical, and health impacts of *Mesona* plant as well as high business competition in common food markets, which mainly produce black jelly in syrup, Indonesian *Mesona* plants, instead of its China alternative, require exploration for further alternation and utilization. Concept of this work involved terms of agreement with the company that expected scientific information of Indonesian *Mesona* plant for its future work. This is the first report to assess nutritional values, total phenolic and flavonoid contents, and antioxidant activities of aqueous extracts of Indonesian *Mesona* plant both with and without addition of sodium carbonate (Na_2CO_3).

Materials and methods

Preparation of sample extracts

Dried Indonesian black cincau (*Mesona* Blume) plant was provided by Chaukuay Songkla Chakangrao Company (Hatyai, Songkhla, Thailand), the main purchaser and distributor in Thailand. The dried whole plant was thoroughly washed twice or thrice with tap water to remove dust and dirt and then divided into two groups that were boiled with Na_2CO_3 (WASC) and without Na_2CO_3 (WOSC). To adhere to the petty patent rule, the ratio of Na_2CO_3 and other critical details are not disclosed in this paper. Each extract group was filtered through a nylon cloth, centrifuged at 7,168× g for 20 min, and the supernatants were tested for nutritional compositions. Each extract was added with 95% ethanol for polysaccharide precipitation at a ratio of 1:1 overnight at 4°C and then centrifuged at 2,800× g for 10 min to obtain supernatant (SWASC and SWOSC) and sediment or precipitate (PWASC and PWOSA). The supernatant was evaporated under reduced pressure at 50–55°C for 30 min to remove alcohol. An overview of the extraction procedure is presented in Figure 1.

Proximate and nutritional composition of Indonesian *Mesona* Blume extract

The WASC and WOSC extracts were evaluated for moisture, ash, crude protein, crude fat, carbohydrate, total dietary fiber, soluble and insoluble dietary fiber, amino acid profiles, fatty acid profiles, minerals, and vitamins at the Central Laboratory (Thailand) Co. Ltd. (Songkhla, Thailand) (ISO/IEC 17025:2017).

Moisture content

Moisture content was determined by Association of Official Analytical Chemist (AOAC, 2019) method 950.46 (B). Briefly, 2 mL of sample was dried and weighed in an aluminium pot by heating at 100–102°C in a hot air oven (FED 115, Binder, Bohemia, NY, USA) for 16–18 h. Then it wascooled down in a desiccator and weighed; the procedure was repeated until a constant weight was obtained.

Ash content

Ash content was determined by AOAC (2019) method 950.14. The heated ash dish was cooled down and weighed. Thereafter, 3–5 mL of the sample was put into an ash dish and heated at 550°C in a muffle furnace (Isotemp 650 Series Model 58; Fisher Scientific, Waltham, MA, USA) until light gray ash was obtained; it was then cooled and weighed. The sample was reheated and reweighed to ensure constant weight. The percentage of ash content was calculated using Equation 1:

$$\text{Ash content } (\%) = \frac{W_3 - W_2}{W_1} \times 100, \quad (1)$$

where W_1 = weight of sample in grams (g), W_2 = weight of crucible (g), and W_3 = weight of crucible + ash (g).

Crude protein content

The Kjeldahl method evaluated crude protein content according to AOAC (2019) method 981.10. In all, 2 mL of sample was weighed and placed in a digested tube containing boiling chips, two catalyst tablets, 15 mL of sulfuric acid, and 3 mL 30–35% hydrogen peroxide. Mixture tubes were heated at 410°C for 45 min in an auto digestor (model Tecator 2508; Foss, Hillerød, Denmark) until a clear solution appeared and cooled. The mixture was transferred to a Kjedahl distillation apparatus (Kjeltec™ 8200; Foss). A receiving flask contained 25-mL boric acid solution with a mixed indicator (green solution). The distillation was initiated immediately and was titrated with

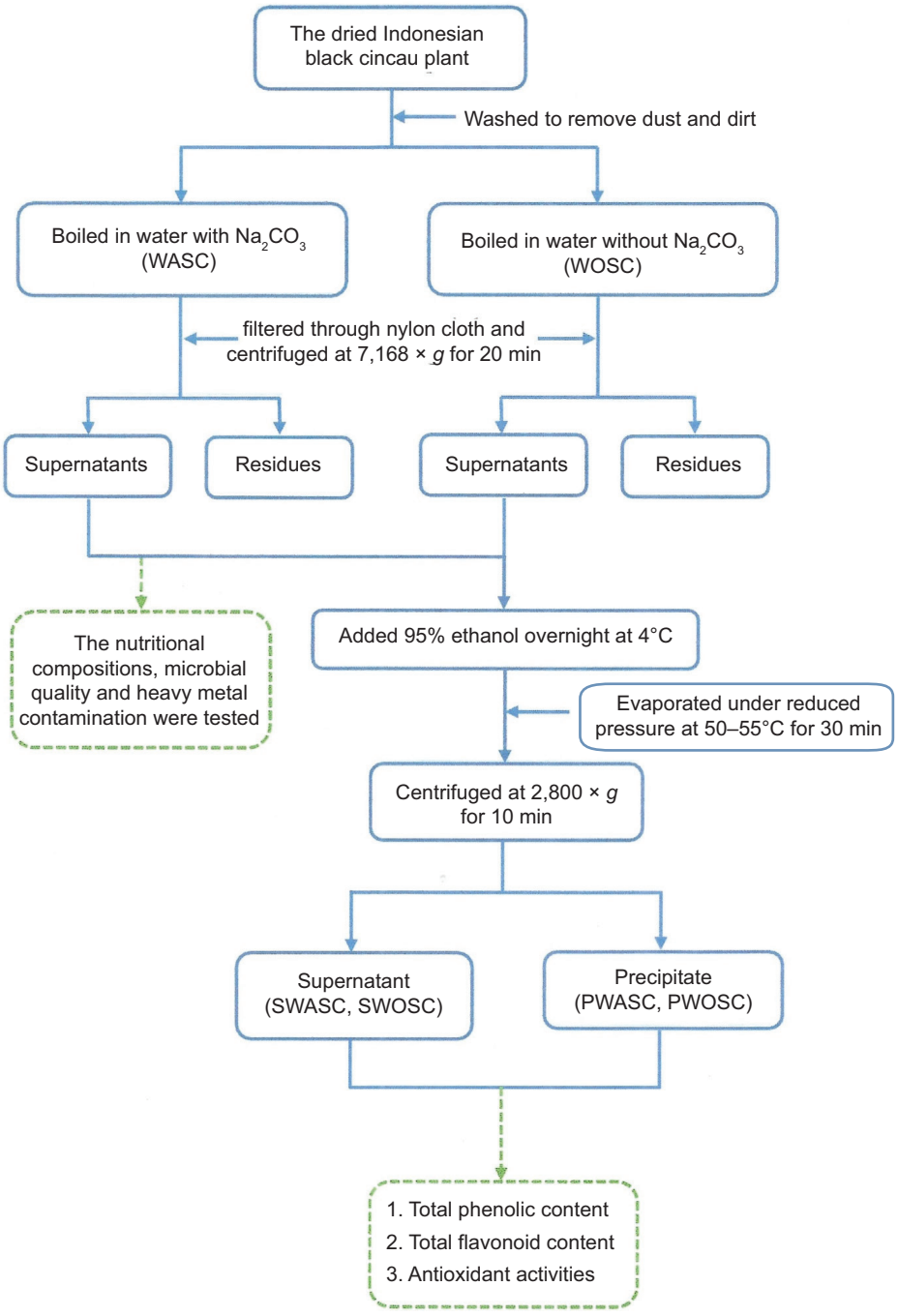

Figure 1. Extraction procedure and evaluation.

0.2-M hydrochloric acid for an end product (gray solution). The percentage of protein content was calculated by Equation 2:

$$\text{Protein (\%)} = (V_A - V_B) \times 1.4007 \times M \times 6.25, \quad (2)$$

where V_A and V_B = volume of HCl used in sample and blank, respectively, 1.4007 = milliequivalent weight N × 100 (%), M = molarity of HCl, and 6.25 = protein factor for meat products (16% N).

Crude fat content

The hydrolysis method (Mojonnier analyzer, EXE1809-04; Moplant, Geldermalsen, The Netherlands) evaluated the fat content according to AOAC (2019) method 948.15. In total, 8-g sample was weighed and 2 mL of hydrochloric acid was mixed in a beaker. The mixture was homogenized (T18 Digital; IKA, Staufen, Germany); then 6 mL of hydrochloric acid was added and heated in a water bath for 90 min and cooled. The mixture was loaded with petroleum ether into a soxhlet

extraction apparatus and dried to obtain a constant weight.

Carbohydrate content (Ellefson, 1993)

The percentage of carbohydrate content was calculated by subtraction using Equation 3:

$$\text{Total carbohydrates (\%)} = 100 - (\text{moisture} + \text{ash} + \text{protein} + \text{fat}). \quad (3)$$

Total dietary fiber

Total dietary fiber was determined by AOAC (2019) method 985.29 (Enzymatic Gravimetric Method). In total, 1-mL sample was weighed and 50-mL phosphate buffer having pH 6.0 was added; the pH was adjusted to 6.0 ± 0.2. The sample was heated at 96–100°C for 15 min in a water bath containing 0.1 mL of Termamyl solution, and cooled to an ambient temperature. The sample was adjusted to reach 7.5±0.2 by adding 0.275-M sodium hydroxide; then 0.1 mL of 50-mg protease in 1-mL phosphate buffer was mixed to the sample. The solution was heated at 60°C for 30 min with constant stirring. After cooling, 10 mL of 0.325-M hydrochloric acid was added to the solution and the pH was readjusted to 4.0–4.6. Then, amyloglucosidase 0.3 mL was added, and the solution was heated at 60°C for 30 min. Thereafter 280 mL of 95% ethyl alcohol was added. The mixture was heated at 60°C for 60 min until precipitate was formed; the mixture was filtered to obtain residue as soluble and insoluble fiber. The residue was used to determine protein and ash contents. Total dietary fiber (TDF) was calculated using Equation 4, while the blank was calculated using Equation 5:

$$\text{Total dietary fiber (TDF)} = \frac{\text{Weight residue} - \text{P} - \text{A} - \text{B}}{\text{Weight test portion}} \times 100 \quad (4)$$

where weight residue = average of weights (in mg) for duplicate blank determinations; P and A = weight (in mg) of protein and ash, respectively; weight test portion = average of two duplicate weights (in mg), and B = blank.

$$\text{Blank (mg)} = \text{Weight residue} - \text{P}_\text{B} - \text{A}_\text{B}, \quad (5)$$

where weight residue = average of weights (in mg) for duplicate blank determinations, and PB and AB = weight (in mg) of protein and ash of blank residues, respectively.

Soluble and insoluble dietary fiber

Soluble and insoluble dietary fiber was evaluated by AOAC (2019) method 991.43. In all, 1-mL sample was weighed and 40-mL MES-TRIS buffer (pH 8.2) was added. Stable heated α-amylase, 50 µL, was added to the solution and stirred gently. Then the mixture was heated in a water bath at 95–100°C for 15 min with constant stirring. The mixture was cooled down to 60°C, and protease solution was added and heated at 60°C for 30 min with constant stirring. The mixture was added with 5-mL 0.561-M hydrochloric acid, and then 1-M sodium hydroxide or hydrochloric acid was added to adjust pH at 4.0-4.7. Amyloglucosidase solution, 300 µL, was mixed with the sample and heated at 60°C for 30 min with constant stirring; the obtained solution was filtered with a filtration flash to obtain a final solution for determining soluble and insoluble dietary fibers. Soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) were calculated using Equation 6. The blank of this method was calculated using Equation 7.

$$\text{B} = \frac{\text{BR}_1 + \text{BR}_2}{2 - \text{P}_\text{B} - \text{A}_\text{B}}, \quad (6)$$

where BR_1 and BR_2 = residue weights (in mg) of blank, and P_B and A_B = weights (in mg) of protein and ash of blank, respectively.

$$\text{SDF and IDF} = \frac{\frac{\text{R}_1 + \text{R}_2}{2} - \text{P} - \text{A} - \text{B}}{\frac{\text{M}_1 + \text{M}_2}{2}} \times 100, \quad (7)$$

where R_1 and R_2 = residue weights (in mg) of duplicate samples; P and A = weights (in mg) of protein and ash, respectively; B = blank weight (in mg); and M_1 and M_2 = weights (in mg) of the sample.

Amino acid profiles

Amino acid profiles were evaluated at the Central Laboratory (Thailand) Co. Ltd. (Songkhla, Thailand) (ISO/IEC 17025:2017) following liquid chromatography-mass spectrometry (LC-MS) as performed by Sarwar *et al.* (1988). The sample was hydrolyzed using 6-N HCl at 110°C for 22 h. Tryptophan, lysine, methionine, and cysteic acid were the oxidized proteins analyzed using performic acid and hydrolyzed using 6-N hydrochloric acid. Lysine was analyzed with sodium borohydride and hydrolyzed using hydrochloric acid. Further, tryptophan was hydrolyzed using 4.2-N sodium hydroxide and centrifuged at 3,000× g for 10 min. The supernatant was injected using column C18, 30 cm (Waters Bondapak, Maple Street Milford, USA). Analytes were detected at set conditions, as 0.2 AUFS (Absorbance unit full scale) and a wavelength of 280 nm. For determining other protein hydrolysates, the sample was diluted using 710-mg disodium hydrogen phosphate containing a mixture of 1-L water-acetonitrile (95:5) and pH was adjusted to 7.40 with phosphoric acid. All analytes and amino acid standards were subjected to an evaporator using nitrogen at 35°C until dry. Methanol was added to dried hydrolysate and redried for 15 min at 35°C before being injected into high-performance liquid chromatography (HPLC). The amino acid profiles were detected by HPLC

(1260 Infinity II LC System; Agilent, Santa Clara, CA, USA). One liter of solvents used for gradient was mixed with 11.45-g sodium acetate and 900-mL water containing 46.5-g acetonitrile and 0.5-mL *triethylamine* (TEA) (solvent A), and 475-g acetonitrile in 410-g water (solvent B). The column was eluted with 100% solvent A at a flow rate of 1 mL/min for 0.5 min. The concentration of solvent B was increased to 46% for the first 9.5 min and then to 100% over the next 0.5 min and was maintained for 1.5 min. The equilibration by solvent A was injected into the analytical column 15 cm at a flow rate of 1.5 mL/min for 7.5 min (Waters Pico-Tag, Maple Street Milford,). For tryptophan determination, 0.01-M sodium acetate was added to 10% acetonitrile and pH was adjusted to 5.8 with glacial acetic acid (solvent A); acetonitrile was mixed in water at a ratio of 60:40 (solvent B). The column was eluted with 100% solvent A at a flow rate of 1 mL/min for 10 min. The column was cleaned with solvent B for 2 min, and equilibration by solvent A was injected into the analytical column at flow rate of 1.5 mL/min for 10 min.

Fatty acid profiles

Fatty acid profiles were evaluated at the Central Laboratory (Thailand) Co. Ltd. (Songkhla, Thailand) (ISO/IEC 17025:2017) using gas chromatography-mass spectrometry (GC-MS) (Choi *et al*., 2023). The sample was hydrolyzed using 10 mL of 8.3-M hydrochloric acid and incubated in a water bath at 70°C for 40 min. Hydrolysates were extracted with 25 mL of ethyl ether in 25 mL of petroleum ether and methylated containing 2 mL of 7% boron trifluoride in methanol and then heated at 100°C for 45 min. Thereafter, the extract was dehydrated with sodium sulfate anhydrous for further analysis. The fatty acid profiles were determined by GC (GC-2010; Kyoto, Japan) equipped with Shimazu GCMS-QP2010 mass spectrometer, and the dehydrate extract was held for 4 min at 100°C in a hot air oven, the heating rate increased from 3°C/min to 240°C and held for 20 min. A volume of 1 µL of sample was injected into Alltech AT™-Silar-90 capillary column (30-m length × 0.25-mm i.d. × 0.2 l µm film thickness; Alltech, Deerfield, IL, USA). Helium was injected as a gas carrier at a flow rate of 0.6 mL/min. The optimal conditions were set as follows: isothermal at 180°C; initial temperature 150°C, held for 10 min, ramp at 5°C/min, final temperature 200°C, held for 2 min; initial temperature 150°C, held for 10 min, ramp at 2.7°C/min; and final temperature 210°C, held for 3 min. At a concentration of 2 mg/mL, 37 mixed *trans* and *cis* fatty acid methyl ester (FAME) standards were dissolved in hexane. Fatty acids of the sample were identified by comparing retention time and mass spectrum with respective standards. The total ion current (TIC) peak area was compared with the standard to calculate the quantification of individual *trans* fatty acids.

Vitamins and minerals determination

Vitamin B_2 and minerals, such as copper, magnesium, manganese, phosphorous, sodium, and zinc, were evaluated at the Central Laboratory (Thailand) Co. Ltd. (ISO/IEC 17025:2017).

Vitamin B2

Vitamin B_2 was evaluated at the Central Laboratory (Thailand) Co. Ltd. (Songkhla, Thailand) (ISO/IEC 17025:2017) by following the HPLC described by Hasan *et al*. (2013). Briefly, 10 mL of the sample was blended and mixed with 25-mL extraction solution (containing 50 mL of acetonitrile in 10 mL of glacial acetic acid and 940-mL deionized water) and incubated in a water bath at 70°C for 40 min with gentle shaking. After cooling, the mixture was filtered and the volume was adjusted up to 50 mL with extraction solution prior to use. As a mobile phase, 20 µL of aliquot was injected into the HPLC using a buffer mixture (hexane sulfonic acid sodium, potassium dihydrogen phosphate, and triethylamine, pH 3.0) and water at a ratio of 96:4 (v/v). C-18 ODS column (250 mm × 4.6 mm, 5 µm; Phenomenex Inc., CA, USA) was set at a temperature of 26°C and the equilibrated solvent was injected into the analytical solvent at a flow rate of 1 mL/min with ultraviolet (UV) detection at 210 nm. The standard solution was prepared and evaluated as mentioned procedure.

Determination of minerals

Minerals were determined according to AOAC (2019) method 984.27. Briefly, the mixture of 1.5 mL of dried sample and 30 mL nitric acid with perchloric acid (2+1) was allowed to stand overnight. The sample was filled into a Kjeldahl flask and heated at low temperatures to remove nitric acid and water. The effervescent reaction ended showing that perchloric acid and organic material had stopped the reaction. The solution was heated at a high temperature for 2 min and then cooled. The solution was added with 20% perchloric acid, diluted with water, and allowed to stand overnight. The sample solution was analyzed for minerals by inductively coupled plasma (ICP) equipped with emission spectroscopy (BRE731400 iCAP PRO ICP-OES; Thermo Scientific, Waltham, MA, USA) using the following wavelengths: copper (324.7 nm), magnesium (285.2 nm), manganese (403.1 nm), phosphorous (214.9 nm), sodium (589.6 nm), and zinc (213.8 nm) and Equation 8:

$$C = A \times \frac{50}{B}, \quad (8)$$

where A = concentration (µg/mL) of element as determined using ICP, B = volume or weight of sample (in mL/g), and C = elemental concentration of the sample solution (in µg/mL or µg/g).

Microbial quality

The microbial quality is the analysis of total viable count (TVC), *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, coliform bacteria, and yeast and molds. Microbial counts were determined at the Central Laboratory (Thailand) Co. Ltd. (Songkhla, Thailand) (ISO/IEC 17025:2017).

Total Viable Count (TVC)

The total viable count was determined according to the method described by Maturin and Peerler (2001). A total of 25 mL of sample paste was mixed with 225 mL of Butterfield's phosphate-buffer and kept in a refrigerator for 30 min. Serial dilutions of 10^{-1}–10^{-5} were prepared using the Butterfield's phosphate-buffer. Appropriate dilution was plated using plate count agar (PCA; Merck, Germany). The plates were separately incubated at 35°C for 48 h. Microbial counts were recorded as colony forming unit/gram of sample (cfu/g).

Determination of Bacillus cereus

Bacillus cereus was analyzed according to the method described by Tallent *et al*. (2020). The sample was prepared using a TVC. Serial dilutions were made and 0.1 mL was spreaded on the surface of Mannitol Egg Yolk Polymyxin Agar (MYP) plates with a sterile glass spreading rod. All the plates were incubated at 30°C for 24 h and a precipitate zone surrounding the colonies was observed. Selected plates contained an estimated 15–150 pink colonies and lecithinase-producing colonies. The bottom of plates into zones were marked with a black felt pen to facilitate counting of colonies that were typical of *B. cereus*. The viable cell was reported as cfu/mL.

Determination of Bacillus subtilis

A slightly modified method, as described by Bartolini and Grau (2019), was applied to *Bacillus subtilis* determination. The sample was prepared using a TVC. Briefly, 0.1 mL of serial dilutions (10^{-5}–10^{-7}) were spread in duplicate with a sterile glass spreading rod in Luria–Bertani (LB) agar plates. These plates were incubated for 24 h at 37°C. The cell counts were recorded as cfu/mL.

Determination of Staphylococcus aureus

The method described by Tallent *et al*. (2016) was applied for *Staphylococcus aureus* analysis. The samples were prepared as described previously with TVC determination; 0.1 mL of serial dilution at 10^{-1}, 10^{-2}, and 10^{-3} were plated on Baird–Parker (BP) agar and incubated at 37°C for 48 h. Typical colonies, black and surrounded with clear zones, were selected to check for plasma clots to indicate *S. aureus*. The viable cell concentration was expressed as cfu/mL.

Determination of coliform bacteria

Coliform bacteria was prepared and determined according to the method described by Feng *et al*. (2020). The sample was prepared using TVC. In all, 1 mL of each dilution was transferred to three Lauryl tryptose tubes (LST) for at least three consecutive dilutions. All LST tubes were incubated at 35°C for 24 h. The tubes were observed for the appearance of gas, or effervescences were recorded and reincubated for another 48 h to confirm a positive test for gas production. The brilliant green lactose bile (BGLB) was inoculated by a loopful of suspension from LST. Gas was produced after incubating at 35°C for 48 h and the tube was recorded to calculate the most probable number (MPN).

Determination of yeast and molds

Yeast and molds were analyzed using the method described by Tournas *et al*. (2001). Samples were added with 0.1% peptone water to achieve 10^{-1} dilution and homogenized to make appropriate dilutions. A quantity of 1 mL of each dilution was aseptically pipeted onto the plates prior to being filled with Dichloran 18% glycerol (DG18) agar; the plates were gently swirled clockwise and anticlockwise. The plates were incubated at 25°C for 5 days in the dark. Viable cells containing 10–150 colonies were counted as cfu/mL.

Determination of aflatoxin

Aflatoxin was determined at the Central Laboratory (Thailand) Co. Ltd. (ISO/IEC 17025:2017) according to the HPLC described in Sun *et al*. (2023). A quantity of 25 mL of samples was mixed with 5-g sodium chloride and methanol in water in a ratio of 7:3; these were homogenized at a high speed for 2 min prior to filtering with a filter paper. The mixture was diluted with 30 mL of water and refiltered through a glass microfiber paper. The dilution sample, 5 mL, was transferred into a syringe barrel with Afla test WB column containing specific antibodies to aflatoxin, further, 5 mL of water was washed and eluted with 1 mL methanol. 2-mL of elution was taken and proper diluted with water before determining HPLC with fluorescence detection (FLD). The 1 L of solvent used as isocratic was a 55:45 v/v mixture of water and methanol. Thereafter, the prepared solvent was injected into 150-mm analytical column XSelect HSS T3 at a flow rate of 1 mL/min. A sample volume of 50 µL was injected into a column equipped with FLD (total run time, 12 min), and the temperature was set at 40°C. The absorbance was recorded at the excitation of 360 nm and emission of 440 nm. Commercial aflatoxin standards, such as B_1, B_2, G_1, and G_2, were prepared with a methanol-water ratio of 50:50 v/v for 1 µg/mL, 0.3 µg/mL, 1 µg/mL, and 0.3 µg/mL respective concentrations. The samples were mixed with a standard (total aflatoxins at an aflatoxin ratio of B_1:B_2:G_1:G_2 = 1:0.3:1:0.3) at 0.5 µg/kg,

4 µg/kg, and 50 µg/kg and homogenized as spike samples. Sample homogenate without standard aflatoxins was set as a blank. The concentration of aflatoxin was calculated using Equation 9:

$$\text{Aflatoxin}(\mu g/kg) = \frac{\text{Spiked sample} - \text{Blank sample}}{\text{Spiked concentation}} \times 100, \quad (9)$$

Determination of heavy metals

Heavy metals were determined at the Central Laboratory (Thailand) Co. Ltd. (ISO/IEC 17025:2017) as described by Omeje *et al*. (2021). A sample volume of 10 mL was blended and homogenized with 60 g of anhydrous sodium sulfate to reduce moisture. The blended sample was extracted using 300 mL of n-hexane at ambient temperature for 24 h. The extract was dried using a rotary evaporator under reduced pressure. A total of 2 g of the extract was transferred into a digestion flask before adding 20 mL of acid containing 650 mL of concentrated nitric acid, 80 mL of perchloric acid, and 20 mL of sulfuric acid and then heated until clear digest was observed. The sample was diluted with deionized water up to 100 mL. The acid of the sample was measured by pH meter. The digested sample was analyzed for heavy metals using pyrolytic-coated graphite tubes with an AAS Spectrophotometer (Varian AA240 spectrophotometer) connected to platform instrument settings and furnace programs. The element standard as reference and blank was prepared and evaluated with duplicated and calculated using Equation 10:

$$C = \frac{a - b}{m} \times v, \quad (10)$$

where C = the concentration of heavy metal (mg/kg), a = the concentration of heavy metal in the sample dilution (mg/L), b = the concentration of heavy metal in the blank (mg/L), v = volume of sample dilution (mL), and m = weight of the sample (g).

Bioactive assessment

Determination of total phenolic content (TPC)

The TPC of SWASC, SWOSC, PWASC, and PWOSC was analyzed using the Folin–Ciocalteu colorimetric assay modified by the method described by Zhou and Yu (2006). Briefly, 20 µL of the sample extract was mixed with 100-µL 10% Folin–Ciocalteu reagent (FCR) and incubated in the dark for 6 min at room temperature. Then, 80-µL of 10% Na_2CO_3 was added and the mixture was allowed to stand in the dark for 30 min at room temperature. The absorbance was measured at 765 nm using an ultraviolet-visible (UV-VIS) spectrophotometer (Varioskan LUX; Thermo Scientific, Singapore). The TPC of the samples was expressed as mg of gallic acid equivalence (mg GAE/g) of dry weight (DW), with a standard curve, $R^2 = 0.9997$.

Determination of total flavonoid content (TFC)

The TFC of SWASC, SWOSC, PWASC, and PWOSC were analyzed using the aluminum chloride colorimetric method with slight modification (Chandra *et al*., 2014). Briefly, 100 µL of the sample was mixed with 100 µl of aluminum chloride and allowed to stand in the dark for 60 min. The absorbance of the mixture was measured at 420 nm using a UV-VIS spectrophotometer (Varioskan LUX; Thermo Scientific). The TFC concentration of the samples was expressed as mg of quercetin equivalence (QE/mg) of dry weight with a standard curve, $R^2 = 0.9992$.

Antioxidant activities

Determination with 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

TheABTS radical cation (ABTS•+) was produced by reacting ABTS solution (7.4 mM) with 2.50-mM potassium persulfate according to the method described by Arnao *et al*. (2001). The mixture was incubated in the dark at room temperature for 12 h before use. The ABTS•+ solution was diluted with distilled water to an absorbance of 1.00. Then 20 µL of aqueous extract or gallic acid standard was added to 280 µL of diluted ABTS•+ solution and allowed to stand in the dark for 2 h. The absorbance was measured at 734 nm. Results were expressed as mg GAE/g DW and reported as an inhibitory concentration (IC_{50}) using Equation 11.

IC_{50} was the initial concentration of an antioxidant required to decrease the initial concentration of a radical by 50%:

$$IC_{50} = \frac{A_C - A\ \text{sample}}{A_C} \times 100. \quad (11)$$

Determination with 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The free radical scavenging was determined using the free radical generator DPPH assay as described by Yen and Hsieh (1997) with slight modifications. Briefly, 100-µL aliquots of SWASC, SWOSC, PWASC, and PWOSC or gallic acid standards were mixed with 100 µL of 0.2-mM DPPH solution. The mixture was kept in the dark for 30 min. The absorbance was measured using a spectrophotometer at 517 nm against a blank of distilled water without DPPH. The results were expressed as mg GAE/g DW and reported as an inhibitory concentration (IC_{50}) calculated using Equation 11.

Determination with ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed following the modified method described by Benzie and Strain (1996). Briefly, 20-mL aliquot of properly diluted extract was mixed with 180-mL FRAP reagent and incubated at 37°C for 30 min. The absorbance was determined at 593 nm against a blank prepared using distilled water. The FRAP solution was freshly prepared by mixing 25 mL of 10-mM 2,4,6-Tris(1-pyridyl)-5-triazine (TPTZ) solution in 40-mM HCl with 25 mL of 20-mM $FeCl_3$ $6H_2O$ and 250 mL of 0.3-M acetate buffer at a pH of 3.6. The results were expressed as mg GAE/g DW and reported as an inhibitory concentration (IC_{50}) calculated using Equation 11.

Statistical analysis

This experiment followed a complete block design (CRD), with data analysis using Duncan's multiple range test. All parameters were determined using three different lots with at least three values. The data were computed by the SPSS Statistics software version 22 (IBM, NY, USA) and expressed as mean ± SD, with $p < 0.05$ considered statistically significant.

Results and Discussion

Proximate and nutritional composition of Indonesian *Mesona* Blume extract

The proximate and mineral compositions of WASC and WOSC are shown in Tables 1 and 2, respectively. All indicated parameter contents of WASC were higher than WOSC. Crude protein content was significantly higher after water extraction with added Na_2CO_3. Thirteen amino acids, such as arginine, histidine, leucine, lysine, methionine, phenylalanine, threonine, alanine, aspartic acid, glutamic acid, glycine, proline, and serine, were found in the extract with added Na_2CO_3, while the control, without added Na_2CO_3, lacked threonine. Sodium carbonate helps to destroy plant cell walls, cytoplasm, and vacuoles to liberate threonine, which is normally linked to phenolics.

Seven fatty acids were found in the extract, such as lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, and alpha-linoleic acid. Both saturated and unsaturated fatty acids found in the extract with added Na_2CO_3 showed a significant increase, compared to the control. Sodium carbonate significantly improved the yield and phytochemical contents. Contents of *Platostoma palustre* or *Mesona chinensis* Benth determined by LC-MS and headspace(HS) GC-MS showed 174 chemical compounds, such as phospholipids, monosaccharides, fatty acids, carboxylic acids, eicosanoids, amino acids, and vitamins. Eleven amino acids included L-dopa, L-proline, L-alanine, L-glutamine, L-phenylalanine, L-threonine, L-(+)-arginine, beta-alanine, L-tryptophan, L-tyrosine, and aspartic acid, while six fatty acids and conjugates included 2-methylpentanoic acid, 3-methylbutanoic acid, 2-methylbutanoic acid, valeric acid, hexadecenoic acid, and palmitic acid (Tang *et al*., 2023).

Liu and Chen (2004) reported 18 amino acids in *Mesona* Blume from China, such as aspartic acid, threonine, serine, glutamic acid, glycine, cysteine, methionine, valine, isoleucine, tyrosine, phenylalanine, lysine, histidine, arginine, proline, alanine, leucine, and tryptophan (Liu and Chen, 2004). *Mesona* plants obtained from Indonesia and used in this study contained different amino acid contents, compared to previous studies. Depending on food flavors, amino acids are divided into three categories: (1) amino acids that provide delicious taste (aspartate, glutamate, 4-aminobutyric, and ornithine), (2) those that provide sweetness (serine, alanine, glycine, and threonine), and (3) that provides bitterness (tyrosine, leucine, valine, methionine, isoleucine, phenylalanine, proline, and lysine) (Hartley *et al.,* 2019; Kim *et al.,* 2017; Shiga *et al.,* 2014). Results indicated that *Mesona* plants used in this study had umami taste, such as that of glutamic acid and aspartic acid.

The nutritional values of *Mesona* plants from different cultivation areas were compared, with results shown in Table 3. Based on the literature review, the amino acid composition of *Mesona* plants from China was higher than that of plants from Indonesia because of variety, topography, climate, fertility, harvesting process, plant adaptation, and storage time. Addition of Na_2CO_3 increased both crude protein and amino acid profiles, with crude protein values lower than carbohydrate content. Regardless of the moisture content, *Mesona* plants mainly contained carbohydrates, followed by ash; however, after addition of Na_2CO_3, content of crude protein improved significantly. Sodium ions (Na^+) and carbonate ions (CO_3^{2-}) during solvation in water generate $CO_3^{2-} + H_2O \rightarrow HCO_3^- + OH^-$, leading to higher pH, which induces precipitation of some extracted compounds, such as amino acids or polysaccharides (Faye *et al.,* 2023). Chethan and Malesshi (2007) reported that addition of sodium hydroxide (NaOH) increased the proportion of minerals in the precipitate. Extraction with Na_2CO_3 or basic agents helps to break down plant cells and liberate various chemical constituents.

Regardless of the extraction process, Indonesian *Mesona* plants mainly contained phosphorus (P) followed by magnesium (Mg), manganese (Mn), and copper (Cu). Addition of Na_2CO_3 increased mineral extraction,

Table 1. Proximate composition of Indonesian *Mesona* Blume extracted with and without sodium carbonate (Na_2CO_3)

Parameter	With Na_2CO_3	Without Na_2CO_3
Moisture (%)	97.92±0.05 [b]	99.34±0.02 [a]
Carbohydrate (%)	0.35±0.04[a]	0.21±0.01[b]
Crude protein (%)	0.17[a]	0.00[b]
Crude fat (%)	0.02±0.02 [a]	0.01±0.01[a]
Ash (g/100g)	0.47±0.01[a]	0.19±0.01[b]
Calories (Kcal/100g)	2.26±0.17[a]	0.93±0.06[b]
Saturated fat (g/100g)	0.01	0.01
Dietary fiber (g/100g)	0.05	0.04
Insoluble dietary fiber (g/100g)	0.03	0.02
Soluble dietary fiber (g/100g)	0.02	0.02
Essential amino acids (mg/100g)		
Arginine	175.35±1.15[a]	122.71±0.17[b]
Histidine	124.88±0.05[a]	117.28±0.03[b]
Leucine	60.24±0.03[a]	55.00±0.06[b]
Lysine	75.59±0.31[a]	70.01±0.11[b]
Methionine	110.65±0.08[a]	105.83±0.01[b]
Phenylalanine	66.72±0.12[a]	56.88±0.06[b]
Threonine	104.67	0.00
Non-essential amino acids (mg/100g)		
Alanine	75.29±0.11[a]	75.25±0.21[a]
Aspartic acid	76.23±0.06[a]	68.24±0.04[b]
Glutamic acid	145.32±0.6[a]	89.65±0.39[b]
Glycine	109.09±0.03[a]	108.72±0.42[a]
Proline	66.80±0.01[a]	60.64±0.16[b]
Serine	39.10±0.14[a]	34.89±0.2[b]
Saturated fatty acids (mg/100g)		
Lauric acid	1.21±0.21[a]	0.49±0.08[b]
Myristic acid	0.80±0.06[a]	0.48±0.10[b]
Palmitic acid	8.60±0.03[a]	3.26±0.23[b]
Stearic acid	3.41±0.11[a]	1.79±0.05[b]
Unsaturated fatty acids (mg/100g)		
Oleic acid	3.55±0.16[a]	1.68±0.13[b]
Linoleic acid	1.42	0.00
Alpha linoleic acid	0.00	0.88

Table 2. Mineral compositions of Indonesian *Mesona* Blume extracted with and without sodium carbonate (Na_2CO_3).

Minerals and Vitamins (mg/100g)	With Na_2CO_3	Without Na_2CO_3
Vitamin B2 (Riboflavin)	0.08	0.05
Cu	0.06±0.00[b]	0.22±0.01[a]
Mg	9.69±0.45[a]	1.72±0.06[b]
Mn	1.49±0.02[a]	0.29±0.00[b]
P	6.29±0.03[b]	8.80±0.11[a]
Na	50.62	0.00
Zn	0.17	0.00

Table 3. Comparison of nutritional values in different cultivation areas from previous studies and in this experiment.

Source	Number of nutritional values		Ref
	Amino acids	Fatty acids	
Indonesia	13	7	This study
Different areas in China	17	–	(Liu and Chen, 2004)
Guangxi, China	11	6	(Su *et al*., 2011)
Jiangxi, China	18	–	(Tang *et al*., 2023)

–: Data not shown.

leading to high mineral contents. Alkaline treatment breaks down ether and ester bonds in lignins or bonds between lignins and hemicelluloses. The rigid structure of the cell wall is weakened and exhibits more porosity, which facilitates higher extractability (Qian *et al.,* 2021). Increased sodium (Na) in the treated group strongly indicated the result of Na_2CO_3 application. Interestingly, Na_2CO_3 liberated zinc (Zn) in *Mesona* plant but it did not happen in boiling water. Therefore, Na_2CO_3 must have a strong effect to solubilize plant cell walls that are made up of cellulose and polysaccharides, making them tough and giving rigidity to plant cells (Alberts *et al.,* 2002). Trace elements, such as Zn, are generally embedded in plant cell walls or are sequestered in vacuoles (Longnecker *et al.,* 1993), indicating that Na_2CO_3 solubilizes cell walls or vacuoles to release Zn. By contrast, alkaline treatment causes the loss of bioactive components, including minerals (Pozo *et al.,* 2010), and a decrease in both Cu and P was noticed. An increase or decrease in compounds indicated the pros and cons of different extraction methods.

Microbial quality, such as total viable count, *Bacillus cereus*, *Bacillus subtilis*, and yeast and molds, of *Mesona* extract with Na_2CO_3 was lower than without Na_2CO_3 because bicarbonate (HCO_3^-) and NaOH generated in water alter the cytosolic pH of bacteria in an extracellular medium caused by proton increment. Addition of Na_2CO_3 led to a higher temperature during extraction through boiling, which helped to destroy bacteria (Metwally *et al.,* 2011). A higher temperature of the sample added with Na_2CO_3 follows Raoult's Law, as more solutes and less free water tends to absorb heat supplied to boil water leading to absorbing more heat energy for vaporization (Kugel, 1998).

Higher media pH induced more erosion, leading to a higher death rate (Teo *et al.,* 1996). Yeast and molds, coliform bacteria, and heavy metals were within the standard criteria of the Thai Food and Drug Administration. The addition of Na_2CO_3 during water extraction reduced

Table 4. Microbial quality, aflatoxin, and heavy metal contamination of Indonesian *Mesona* Blume with and without sodium carbonate

Parameter	With Na_2CO_3	Without Na_2CO_3	Standard regulation (Thai FDA, ready to eat pasteurized herbal products)
1. Microbial quality			
Total viable count (CFU/ml)	$4.9x10^4$	$3.2x10^5$	$<5x10^7$
Bacillus cereus (CFU/ml)	1.0x10	1.5x10	<100
Bacillus subtilis (CFU/ml)	<10	2.9x10	–
Staphylococcus aureus (CFU/ml)	No	No	<100
Yeast and mold (CFU/ml)	3.0	2.0x10	$<5x10^2$
Total coliforms MPN/ml	<0.3	<0.3	<3
2. Aflatoxin			
Aflatoxin B_1 (µg/kg)	No	No	No
Aflatoxin B_2 (µg/kg)	No	No	No
Aflatoxin G_1 (µg/kg)	No	No	No
Aflatoxin G_2 (µg/kg)	No	No	No
Total aflatoxin (µg/kg)	No	No	No
3. Heavy metal			
Cadmium (mg/kg)	<0.008	<0.008	<0.3
Lead (mg/kg)	<0.050	<0.050	<10

No: Not detected.
–: Not defined.

the microbial load, with both CO_2 generation and basic condition of pH 7.87 during heating having a stronger cell-destroying effect. Therefore, using Na_2CO_3 and boiled water combine to inhibit and kill bacteria via the multitasking of bicarbonate (HCO_3^-), NaOH, and CO_2 as well as heat. Aflatoxins and *Staphylococcus aureus* were not found, as shown in Table 4. Cognizance of *Bacillus* spp. should be further investigated due to the nature of dried plant material, which is easily contaminated with spore-forming bacteria having high resistance, even in harsh conditions (Soni *et al.*, 2016; Tirloni *et al.*, 2022).

Total phenolic content

The TPC of SWASC (83.47±0.19 mg GAE/g DW), SWOSC (68.04±0.31 mg GAE/g DW), PWASC (57.15±0.71 mg GAE/g DW), and PWOSC (32.82±0.16 mg GAE/g DW) was determined by the Folin–Ciocalteu method (Figure 2). The TPC of supernatant treated with Na_2CO_3 was higher, compared to the TPC of supernatant without Na_2CO_3 because more free phenolic compounds were liberated during water extraction. Polyphenolics in supernatants play a key role in TPC. PWASC and PWOSC, which mainly contained polysaccharides, had lower TPC values, indicating fewer active groups to react with the Folin–Ciocalteu reagent. It indicated that alkalinity of Na_2CO_3 helped to solubilize and hydrolyze the cell walls or polysaccharide structures to release phenolic compounds or certain phytochemicals having fucntional group, resulting in higher TPC and antioxidant

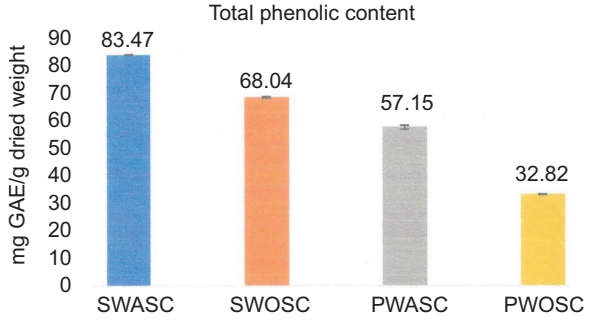

Figure 2. Total phenolic content and antioxidant activity impacted by Na_2CO_3.

activities. Comparison of the present study with other research works, as shown in Table 5, discovered that the yield of TPC in the extract with Na_2CO_3 was 83.47±0.19 mg GAE/g DW, compared to TPC in the extract without Na_2CO_3, that is 68.04±0.31 mg GAE/g DW, which was almost 3.5 times lower, compared with the findings of Chusak *et al.* (2014). However, the TPC value obtained in the present study was also comparable with some studies (Table 5). In general, a lower TPC and antioxidant activity in Indonesian *Mesona* plant appeared to have a lower quality, compared with Chinese *Mesona* plant. This information helped purchaser to deliver data to farmers and the industry to further improve quality and process as well as product specification. In addition, literature

Table 5. Comparison of TPC and TFC in different areas from previous studies and in this experiment.

Sample source	Water extraction		TPC (mg GAE/ g extract)	TFC (mg QE/ g extract)	Ref
	With Na_2CO_3	Without Na_2CO_3			
Indonesia		/	68.04±0.31	3.66±0.10	This study
Indonesia	/		83.47±0.19	6.02±0.09	This study
Herbal Drugstore in Bangkok, Thailand		/	212.37± 5.64	23.44±2.50 mg CE/g extract	(Chusak *et al*., 2014)
Local market in Thailand		/	24.20 ± 1.35 mg GAE/mL extract	–	(Wongverawattanakul *et al*., 2022)
China Source 1		/	68.63±4.69	3.49±0.46	(Suriyaphan *et al*., 2023)
China Source 2		/	133.27±8.06	5.83±0.38	(Suriyaphan *et al*., 2023)
Indonesia		/	112.32±1.50	4.42±0.22	(Suriyaphan *et al*., 2023)
Vietnam		/	126.65±5.46	5.24±0.68	(Suriyaphan *et al*., 2023)

–: Data not shown.

review and the present result proved that alkaline hydrolysis (pH > 7) strongly helped the liberation of bound phenolic compounds from cell wall polysaccharides (Sun *et al.,* 2002). Conidi *et al.* (2020) discovered that using pH > 7 provided slightly higher yield of total polyphenols, compared to using lower pH values. Generally, ester bonds were broken down to release phenolic compounds with alkaline hydrolysis (Mendez-Lagunas *et al.,* 2020; Zhong *et al.,* 2022). Interestingly, TPC and TFC in the present study were equal to or lower than that in other studies. These diverse values resulted from different plant samples, storage periods, concentration of Na_2CO_3, boiling or extraction time as well as assay preparation methods. These conditions should be further investigated and controlled to maintain quality assurance.

Total flavonoid content

The TFC of SWASC, SWOSC, PWASC, and PWOSC was evaluated by the flavonoid-aluminum chloride conjugation method (Figure 3). Results showed that SWASC had maximum TFC (6.02±0.09 mg QE/g DW), while the TFC of SWOSC, PWASC, and PWOSC decreased gradually as 3.66±0.10, 1.95±0.05, and 0.43±0.01 mg QE/g DW, respectively. Suriyaphan *et al.* (2023) reported that *Mesona chinensis* extract imported from Indonesia had higher TPC (112.32±1.50 mg GAE/g DW) and TFC (4.42±0.22 mg QE/g DW), compared to our results. These different biological values were due to material sources, extraction methods, and cultivation environments (Li *et al.,* 2021; Yan *et al.,* 2019). Ghasemzadeh *et al.* (2011) reported the highest TFC in methanolic extracts, compared to other polar solvents (acetone and chloroform), suggesting that increased TFC was solvent polarity-dependent, while Rommel and Wrolstad (1993) found that TFC was significantly reduced after boiling. Some flavonoid compounds may be heat labile or lose their function because of the breaking of bonds (Chaaban *et al.,* 2017). *Mesona* plants generally contained higher TPC than TFC.

Antioxidant activities

The antioxidant activity of SWASC, SWOSC, PWASC, and PWOSC extracts based on the ABTS, DPPH, and FRAP free radical scavenging assays were reported as mg GAE/g DW (Figure 4) and IC_{50} value (Figure 5). The results exhibited antioxidant activity ($p < 0.05$) in the order of SWASC < SWOSC <PWASC < PWOSC, as shown in Figures 4 and 5. Generally, antioxidant activity is determined and expressed as a standard equivalent and/or IC_{50}. Results indicated that determining of both antioxidant activity and IC_{50} functioned harmoniously. However, recently, using of a standard equivalent must be more accepted and is precise, compared to IC_{50}, because of a common mistake in the unit used and the concentration, which is commonly created to express IC_{50} (de Menezese *et al*., 2021). In additon, concentration of the antioxidant is widely misused with a total disregard for DPPH• concentration, while the molar ratio of antioxidant–DPPH• would be the correct application (de Menezese *et al*., 2021).

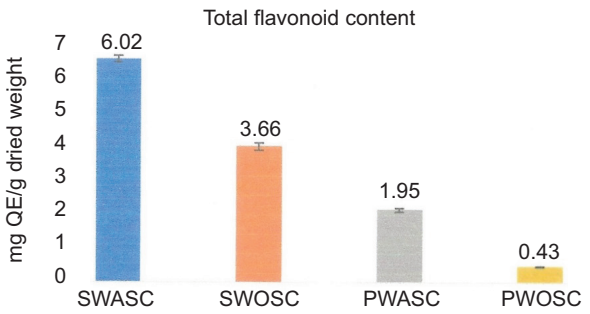

Figure 3. Total flavonoid content.

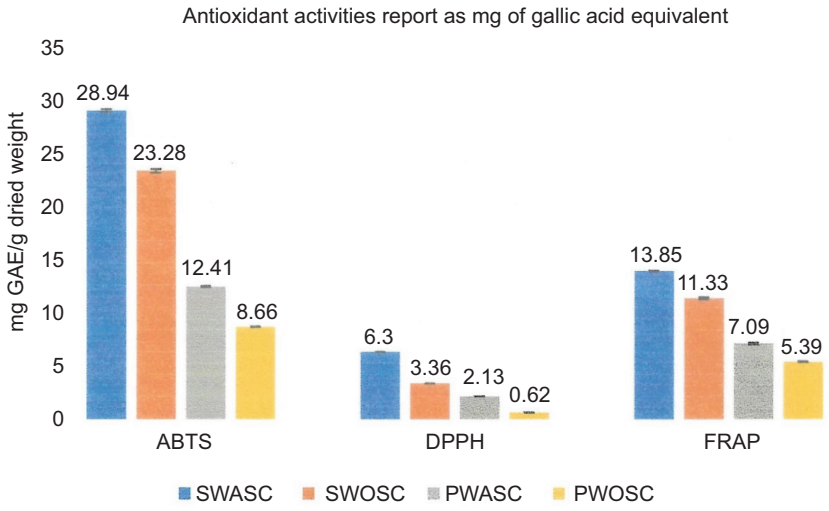

Figure 4. Antioxidant activity was reported as mg GAE/g DW.

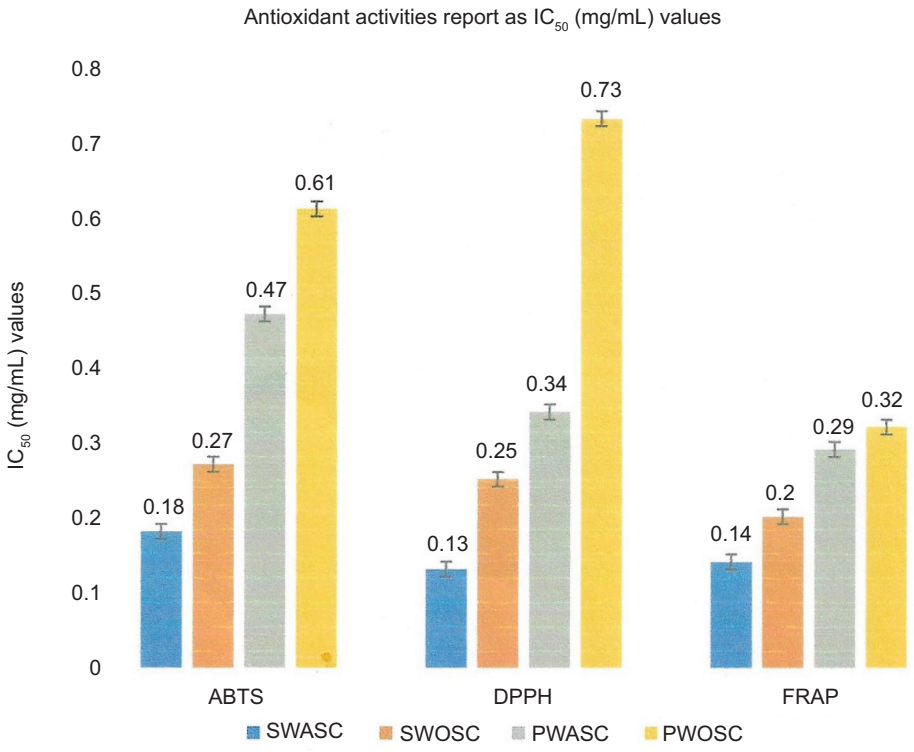

Figure 5. Antioxidant activity was reported as IC_{50} (mg/mL) values.

In all antioxidant activity assays, the extract of SWASC revealed stronger antioxidant activity than other samples, which highly correlated with TPC and TFC (R^2 =0.9976 and 0.9966, respectively). This finding indicated that the Indonesian *Mesona* Blume extract treated with an alkaline solution showed higher TPC, TFC, and antioxidant activity than the extract without an alkaline solution. The precipitated extracts as polysaccharides showed lower antioxidant activity than supernatants. This result confirmed that polysaccharides, as a main compound in the precipitate, expressed less antioxidation, compared to the supernatant which contained more phenolics. TPC and TFC played a significant role in the Indonesian Mesona plant extract, particularly in the sample treated with Na_2CO_3, while carbohydrates as the main compound of the plant also showed effects.

Conclusion

Indonesian *Mesona* Blume extraction with Na_2CO_3 yielded increased essential amino acids (26.52%), non-essential amino acids (14.54%), essential fatty acids (57.06%), nonessential fatty acids (48.49%), and minerals (83.06%), compared to samples without added Na_2CO_3. TPC, TFC, and antioxidant activity increased significantly in the supernatant, followed by the precipitate of the samples treated with Na_2CO_3. Supernatant added with Na_2CO_3 during extraction yielded higher TPC (18.49%), TFC (39.20%), ABTS (17.28%), DPPH (42.86%), and FRAP (18.19%), compared to the samples without added Na_2CO_3. Similarly, the precipitated sample with added Na_2CO_3 showed higher TPC (42.57%), TFC (77.95%), ABTS (46.33%), DPPH (70.89%), and FRAP (23.98%), compared to the samples without added Na_2CO_3. Addition of Na_2CO_3 assisted the extraction process and yielded higher bioactive compounds in Indonesian *Mesona* Blume. TPC, TFC, and antioxidant properties were mainly found in supernatants as phenolic compounds and not carbohydrates. Results of the present study provide essential information to the industry to plan purchase of raw material and control of production. However, other health functions, such as antihyperglycemic and gut microbiota, should also be assessed to better understand and utilize Indonesian *Mesona* plants.

Acknowledgment

The authors extended their appreciation to the National Research Council of Thailand (NRCT) and Chinese Vegetable Jelly Songkhla Chagungrao Co. Ltd. (N23G660028).

Declaration of Interest

The authors declared no conflict of interest.

Availability of Data

Data are made available on request.

Author Contributions

Conceptualization, S.S.; methodology, R.S., S.S., S.W. and W.U.; validation, R.S., S.S., S.W. and W.U.; formal Analysis, R.S.; investigation, R.S.; resource, R.S., S.S., S.W. and W.U.; data curation, R.S., S.W. and W.U.; writing-original draft preparation, R.S.; writing-Review & Editing, R.S., S.S., S.W. and W.U.; supervision, S.S.; project administration, S.S.; funding acquisition, S.S. All authors have read and agreed to the published version of the manuscript.

Funding

The authors declared that this study received funding from Chinese Vegetable Jelly Songkhla Chagungrao Co. Ltd. and the National Research Council of Thailand (NRCT) under PhD student program. The funder was neither involved in the study design, collection, analysis, and interpretation of data nor in the writing of this article or the decision to submit it for publication.

Conflicts of Interest

The authors declare no conflict of interest.

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