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Research Article

Evaluation of the Use of Different Solvents for Phytochemical Constituents, Antioxidants, and *In Vitro* Anti-Inflammatory Activities of *Severinia buxifolia*

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Severinia buxifolia (Rutaceae) is a promising source of bioactive compounds since it has been traditionally used for the treatment of various diseases. The present study aimed at evaluating the impact of different solvents on extraction yields, phytochemical constituents and antioxidants, and *in vitro* anti-inflammatory activities of *S. buxifolia*. The results showed that the used solvents took an important role in the yield of extraction, the content of chemical components, and the tested biological activities. Methanol was identified as the most effective solvent for the extraction, resulting in the highest extraction yield (33.2%) as well as the highest content of phenolic (13.36 mg GAE/g DW), flavonoid (1.92 mg QE/g DW), alkaloid (1.40 mg AE/g DW), and terpenoids (1.25%, w/w). The extract obtained from methanol exhibited high capacity of antioxidant (IC_{50} value of 16.99 μg/mL) and *in vitro* anti-inflammatory activity (i.e., albumin denaturation: $IC_{50} = 28.86$ μg/mL; antiproteinase activity: $IC_{50} = 414.29$ μg/mL; and membrane stabilization: $IC_{50} = 319$ μg/mL). The antioxidant activity of the *S. buxifolia* extract was found to be 3-fold higher than ascorbic acid, and the anti-inflammatory activity of *S. buxifolia* extract was comparable to aspirin. Therefore, methanol is recommended as the optimal solvent to obtain high content of phytochemical constituents as well as high antioxidants and *in vitro* anti-inflammatory constituents from the branches of *S. buxifolia* for utilization in pharmacognosy.

1. Introduction

Oxidative stress and autoxidation of human lipids and lipoproteins result in the formation of potentially toxic compounds, causing various human health problems such as aging, cardiovascular disease, diabetes, and cancer [1, 2]. To solve these problems, synthetic antioxidants, such as propyl gallate, butylated hydroxyanisole, tert-butylhydroquinone, and butylated hydroxytoluene, have been extensively used to avoid the oxidative stress and autoxidation process [3]. Nevertheless, the use of such compounds is restricted due to their side effects on human health; therefore, efforts have been undertaken to search for natural agents as alternatives to synthetic antioxidants [3, 4]. As a result, the use of natural antioxidants from plants has attracted considerable attention in recent years because plants are one of the best sources of natural antioxidants such as flavonoid, phenolic, and alkaloid compounds [3, 5]. These compounds have ability to quench free radicals, chelate catalytic metals, and scavenge oxygen [3, 5]. These antioxidant agents have been proven to be a promising medicinal product to prevent oxidative stress, disease, and maintain health and delay aging processes. In addition, these natural antioxidants are recognized as potential anti-inflammatory agents, which safely protect the human body against inflammation, thus preventing diseases and disorders caused by inflammation [6–8]. Therefore, finding new and safe natural agents with antioxidant and anti-inflammatory activity is the objective of the continued study.

Severinia buxifolia (*Atalantia buxifolia*), which belongs to the Rutaceae family, is an evergreen citrus plant native to China, Cambodia, Laos, Vietnam, and Taiwan [9, 10]. *S. buxifolia* is used as a traditional medicine to treat cough, snakebites, malaria, paralysis, traumatic swelling, chronic rheumatism, and pain [11, 12]. The health benefits of *S. buxifolia* are attributed to phytochemical constituents that have physiological actions on the human body [12, 13]. Various bioactive compounds are found from the branches and roots of *S. buxifolia* including acridone alkaloids, tetranorterpenoids, coumarins, limonoids, and sesquiterpenes [11, 12]. These compounds possess various biological activities such as antifeedant, antiallergic, antibacterial, antimalarial, antivirus, antioxidant, and anti-inflammatory activities [12, 14]. *S. buxifolia* is therefore considered to be a promising source of antioxidant and anti-inflammatory agents.

Extraction is the main process by which bioactive compounds may be obtained from biomass materials. The objective of extraction process is to maximize the amount of target compounds and to obtain the highest biological activity of these extracts [15]. The extraction yield and biological activity of the resulting extract is not only affected by the extraction technique but also by the extraction solvent [16, 17]. Many solvents, including methanol, ethanol, acetone, and water, have been used for extracting bioactive compounds from the plant material. Due to the variety of bioactive compounds contained in plant materials and their differing solubility properties in different solvents, the optimal solvent for extraction depends on the particular plant materials, and the compounds that are to be isolated [16, 17]. Therefore, recommendation of suitable extraction solvent for individual plant materials is generally difficult. For *S. buxifolia*, most research has focused on the screening and identification of bioactive compounds. However, no study has reported the effect of solvent on the bioactive compounds extraction from *S. buxifolia* and the biological activity of the extracts.

The present study examined the effect of distilled water and organic solvents (methanol, ethanol, chloroform, dichloromethane, and acetone) on the extraction yield and the content of phenolics, alkaloids, and flavonoids. The antioxidant and anti-inflammatory activities of the resulting extracts were also investigated.

2. Materials and Methods

2.1. Chemicals. Methanol (HPLC grade) (MeOH), ethanol (99%) (EtOH), chloroform (HPLC grade) ($CHCl_3$), dichloromethane (HPLC grade) (CH_2Cl_2), acetone (HPLC grade) (Me_2CO), Folin-Ciocalteu, quercetin, gallic acid, ascorbic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and other reagents were analytical grade (HPLC grade) was provided by Sigma-Aldrich, Singapore.

2.2. Identification of Plant Material. The branches of *S. buxifolia* were collected from Phu Loc district (Thua Thien Hue province, Vietnam). The plants were taxonomically identified by the Botany Research and Development Group of Vietnam (Vietnam). The branch materials were obtained by removing the leaves and thorns, washing under running tap water, and drying at 60°C for 72 h. The resulting dried sample was ground using a mill (Jehmlich, Germany) and used for further experiments.

2.3. Preparation of Plant Extracts. A comparative study was performed to investigate the effect of solvents on the extraction yield and the content of phenolics, flavonoids, alkaloids, and terpenoids. The *S. buxifolia* extract was prepared according to protocol of Do et al. [4] with some minor modifications. Plant samples (1 g) were immersed in different solvents (distilled water (H_2O), MeOH, EtOH, $CHCl_3$, CH_2Cl_2, and Me_2CO) at a sample : solvent ratio of 1 : 20 (w/v) for 24 h at 60°C. The mixtures were then homogenized at 60°C for 4 h using a homogenizer (IKA, Germany). The extracts were then filtered using the filter paper, concentrated at 60°C using a rotary evaporator (Polylab, India) and freeze-dried for 24 h. All freeze-dried extracts were stored at 4°C prior to further experiments. All experiments were repeated in triplicate.

2.4. Determination of Extraction Yield. The extraction yield (%) was calculated as follows:

$$\text{extraction yield (\%)} = \frac{\text{weight of the extract after evaporating solvent and freeze drying}}{\text{dry weight of the sample}} \times 100. \quad (1)$$

2.5. Total Phenolic, Flavonoid, Alkaloid, and Terpenoid Content Determination. The freeze-dried extract (1 g) obtained by each solvent was dissolved in absolute ethanol (1 : 10, w/v) and subsequently used for phenolic, flavonoid, alkaloid, and terpenoid determination.

2.5.1. Total Phenolics (TPC). The phenolic level was determined by the modified Folin-Ciocalteu assay, as described by McDonald et al. [14]. One milliliter of each extract and 5 mL of 10% Folin-Ciocalteu reagent were mixed, and then 4 mL of 2% Na_2CO_3 was added to the mixture. Reagent without extract (only absolute ethanol) was used as a control. After incubating all samples at room temperature for 60 min, their absorbance was measured at 765 nm using the V-730 UV-Vis spectrophotometer (Jasco, USA). The calibration curve for gallic acid (0–100 μg/mL) was established to calculate phenolic content. Total phenolics (TPC) were shown as the mg gallic acid equivalent (GAE) per gram of extract (dry weight). The equation of calibration curve was $y = 0.0066x - 0.0135$, where $R^2 = 0.9996$.

2.5.2. Total Flavonoids (TFC). Total flavonoids were determined using the modified aluminum chloride colorimetric method of Chang et al. [15]. Briefly, the mixture of 2 mL extract and 0.5 mL of 5% $AlCl_3$ and 0.5 mL of 1 M potassium acetate solution was incubated at room temperature for 15 min. Absolute ethanol was used as a control. The absorbance of all samples was then measured at 415 nm using a V-730 UV-Vis spectrophotometer (Jasco, USA). Quercetin was used as a reference standard to calculate flavonoid content. Total flavonoid content (TFC) was shown as mg of quercetin equivalent (QE) per gram of extract (dry weight). The equation of standard curve was $y = 0.0289x + 0.1722$, where $R^2 = 0.995$.

2.5.3. Total Alkaloids (TAC). Total alkaloids were measured by the colorimetric method of Ajanal et al. [16]. One milliliter of plant extract was washed 3 times with 10 mL chloroform before adjusting the pH to neutral. The extract was then mixed with 5 mL of phosphate buffer and 5 mL of bromocresol green solution. The mixture was then vigorously shaken with chloroform and collected in a 10 mL volumetric flask. The absorbance of the solution was measured at 470 nm using a spectrophotometer (V-730 UV-Vis Spectrophotometer, Jasco, USA). Atropine was used as the reference standard to calculate the alkaloid content. Total alkaloid content (TAC) was calculated as mg of atropine equivalent (AE) per gram of extract (dry weight). The equation of standard curve was $y = 0.0031x + 0.028$, where $R^2 = 0.9952$.

2.5.4. Total Terpenoids (TTeC). Total terpenoid content in the crude extracts of *S. buxifolia* branches was determined by the method as described by Ferguson [18] with some small modifications. After soaking in absolute ethanol for 24 h, the extract was filtered and then the filtrate was extracted with petroleum ether using separating funnel. The ether extract was taken as the measure of total terpenoids (TTeC):

$$\text{total terpenoid content } (\%) = \left[\frac{\text{final weight of the sample} - \text{initial weight of the extract}}{\text{weight of the sample}}\right] \times 100. \quad (2)$$

2.5.5. High-Performance Liquid Chromatography Technique Analysis for Acridone Alkaloid Determination. Acridone alkaloids were determined using a high-performance liquid chromatography (HPLC-2160, Aligent, USA) equipped with a UV detector (Agilent Series 1100) and an Eclipse Plus C18 column (4.6 × 250 mm). Methanol-acetonitrile (20/80, v/v) at the flow rate of 0.5 mL/min was used as the mobile phase. The temperature of the column was set at 25°C. 20 μL of the sample dissolved in MeOH (20–100 ppm), was injected into the column, and the detection was attained at 250 nm. 9(10H)-Acridanone (Sigma) at the concentration of 0.5 mg/mL was used as the reference standard. The percentage of the compound in the plant extract was calculated using the following equation [19]:

$$\text{percentage of the compound} = \left[\frac{\text{AUC of the plant sample/AUC of the standard}}{\text{wt. of the plant used in extraction}}\right] \times C \times D \times 100, \quad (3)$$

where wt. is the weight of plant used in extraction; C corresponds to concentration of standard used in HPLC; D is dilution factor; and AUC corresponds to area under curve.

2.6. Determination of Antioxidant Activity. The antioxidant activity of the *S. buxifolia* extracts was determined using DPPH-free radical scavenging assay described by Mahdi-Pour et al. [17] with slight modifications. The extract was serially diluted to concentrations of 25, 50, 100, 200, and 500 μg/mL. One mL of each dilution was mixed with 1 mL of DPPH solution (0.004% in ethanol) and incubated at 37°C for 30 min. The absorbance of mixture was then measured at 517 nm using a spectrophotometer (V-730 UV-Vis Spectrophotometer, Jasco, USA). Absolute ethanol was used as a negative control. The DPPH scavenging activity was calculated as follows:

$$\text{DPPH scavening activity}\,(\%) = \frac{A_0 - A}{A_0} \times 100, \quad (4)$$

where A is the absorbance of sample containing extract and A_0 is the absorbance of the negative control (0.004% DPPH solution).

Ascorbic acid was used as a positive control. The concentration required for a 50% inhibition of DPPH (IC_{50}) was then calculated by plotting the percentage of residual DPPH against the sample concentration.

2.7. Determination of In Vitro Anti-Inflammatory Activity. The *in vitro* anti-inflammatory activity of the *S. buxifolia* extracts was established by the assessment of inhibition of albumin denaturation, membrane stabilization, and anti-proteinase activity, as described by the previous studies with slight modifications [20, 21]. The freeze-dried extract obtained from each solvent was serially diluted in dimethyl sulfoxide (DMSO) from 25 to 500 μg/mL. Aspirin (100 μg/mL, Sigma-Aldrich, Singapore), a standard anti-inflammatory drug, was used as a positive control, whereas DMSO was used as a negative control.

2.7.1. Inhibition of Albumin Denaturation. To prepare the reaction mixture, 1 mL of 1% aqueous solution of bovine albumin fraction was added into 1 mL of tested extract. The pH of reaction mixture was then adjusted to 6.3 before being incubated for 20 min at 37°C and heated to 51°C for 30 min. After cooling to room temperature, the absorbance of the sample was measured at 660 nm using a V-730 UV-Vis spectrophotometer (Jasco, USA). The percentage inhibition of protein denaturation was calculated using the following equation, and the results were reported as IC_{50} values (concentration required for a 50% inhibition):

$$\text{percentage inhibition}\,(\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100, \quad (5)$$

where A_{control} is the absorbance of negative control (DMSO) and A_{sample} is the absorbance of the extract.

2.7.2. Antiproteinase Activity. One mL sample was added into reaction mixture containing 1 mL of 20 mM Tris HCl buffer (pH 7.4) and 0.06 mg trypsin. The mixture was then incubated for 5 min at 37°C before adding 1 mL of 0.7% (w/v) casein. The reaction mixture was incubated for an additional 20 min and 2 mL of 70% perchloric acid ($HClO_4$) was subsequently added to stop the reaction. The reaction mixture was centrifuged at 4°C, 6,000 rpm, for 10 min to collect the supernatant. The supernatant was measured the absorbance at 210 nm using a spectrophotometer (V-730 UV-Vis Spectrophotometer, JASCO, USA). The percentage inhibition of proteinase was calculated using the following equation, and the results were reported as IC_{50} values:

$$\text{percentage inhibition}\ (\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100. \quad (6)$$

2.7.3. Membrane Stabilization. A blood sample was obtained from a healthy human volunteer who did not use any nonsteroidal anti-inflammatory drugs for 2 weeks. The blood cells were centrifuged at 3000 rpm for 10 min, washed with normal saline (3 times), and reconstituted as 10% suspension in normal saline. To perform the heat-induced haemolysis assay, 1 mL of sample was mixed with 1 mL of 10% RBC suspension and incubating for 30 min at 56°C. The mixture was then cooled and centrifuged at 2500 rpm for 5 min to obtain the supernatant. The supernatant was then measured the absorbance at 560 nm using a spectrophotometer (V-730 UV-Vis Spectrophotometer, Jasco, USA). The percentage inhibition of haemolysis was calculated using the following equation, and the results were reported as IC_{50} values:

$$\text{percentage inhibition}\ (\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100. \quad (7)$$

2.8. Statistical Analysis. All analyses were done at least in triplicate, and these values were then showed as mean values along with their standard derivations (±SD). Minitab software was used to analyze data. Statistical comparisons were carried out by analysis of variance (ANOVA) Tukey's multiple comparison test, and p values < 0.05 were considered as significant. Principal component analyses (PCAs) were performed on a dataset containing the content of each chemical component group (phenolics, flavonoids, alkaloids, and terpenoids) as well as the inhibitory value of biological activities (antioxidant and *in vitro* anti-inflammation) of crude extracts of *S. buxifolia* branches. Principal components (PCs) were calculated using a correlation matrix.

3. Results

3.1. Effects of Different Solvents on Extraction Yield. Water and organic solvents (methanol, ethanol, chloroform, dichloromethane, and acetone) were studied for their effects on the extraction yield of *S. buxifolia*. Results showed a significant difference in the extraction yield using different solvents. Among solvents tested, methanol resulted in the highest extraction yield (33.2%), followed by distilled water (27.0%), ethanol (12.2%), acetone (8.6%), chloroform (7.2%), and dichloromethane (4.9%) (Figure 1), indicating that the extraction efficiency favors the highly polar solvents.

3.2. Effects of Solvents on Phenolic, Flavonoid, Alkaloid, and Terpenoid Content. The influence of tested solvents on crude extracts of *S. buxifolia* branches is depicted in Figure 2 and Table 1. A significant difference in the content of bioactive components (i.e., phenolics, flavonoids, alkaloids, terpenoids) in the *S. buxifolia* extracts was achieved in the present study ($p < 0.05$; Table 1). The principal component analysis (PCA) confirmed variations in the content of such compounds in the extracts of *S. buxifolia* branches using different solvents (Figure 2). PCA using the content of the individual phytochemical constituent captured 92.0% of the total variance on a score plot constructed with the two first PCs (PC1 76.5% and PC2 15.5%). Most of solvent extractions clustered on one side of the scatter plot except MeOH and EtOH (Figure 2). As shown in Figure 2, MeOH was highly separated from other tested solvents.

The effect of different solvents on chemical components of *S. buxifolia* branches was also analyzed by ANOVA. The results showed that methanol exhibited the optimal solvent to extract the bioactive components from *S. buxifolia* branches ($p < 0.001$) since the highest content of phenolics (13.36 mg GAE/g DW), flavonoids (1.92 mg QE/g DW), alkaloids (1.40 mg AE/g DW), and terpenoids (1.25%, w/w) were obtained by using this solvent. Distilled water also showed high efficiency in the extraction of phenolic compounds with 5.95 mg GAE/g DW of phenolics being extracted. However, low levels of flavonoids, alkaloids, and terpenoids were obtained in the water extracts. Different from distilled water, although ethanol resulted in lower phenolic content (3.60 mg GAE/g DW) than distilled water (5.95 mg GAE/g DW), this solvent extracted higher alkaloids (1.34 mg AE/g DW) as well as terpenoids (0.97%, w/w) in comparison with distilled water extract (0.16 mg AE/g DW and 0.43%, w/w, respectively). The present study also found

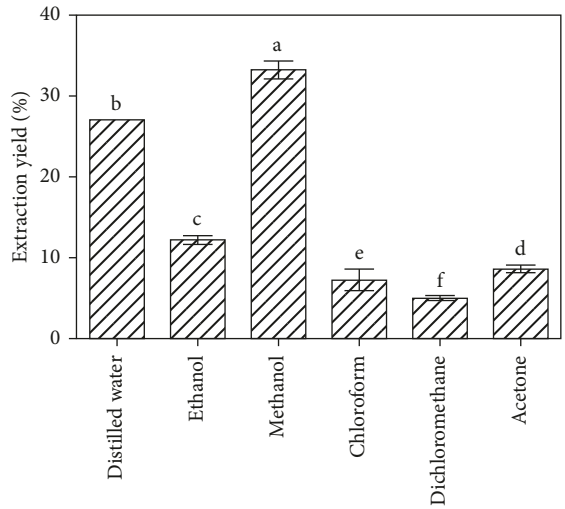

FIGURE 1: Effect of different solvents on extraction yield. Vertical bars illustrate the standard deviation ($n = 3$). Different lowercase characters represent significant difference at $p < 0.05$ by Tukey's multiple range test.

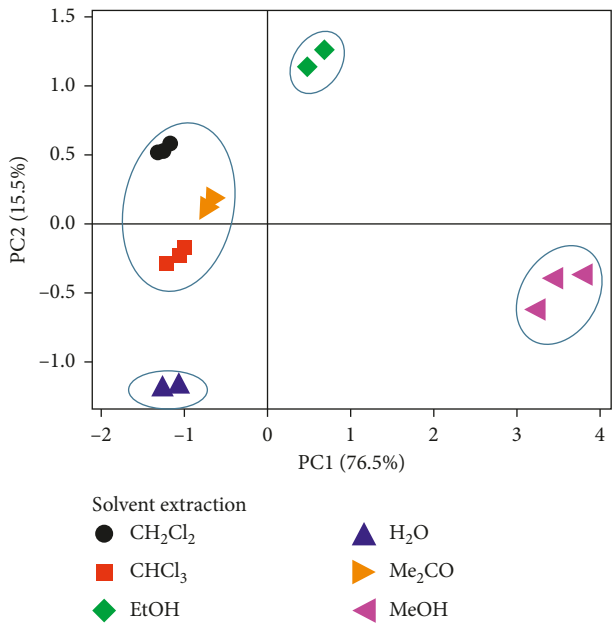

FIGURE 2: Principal component analysis of the content of phytochemical components from *S. buxifolia* extracted by different solvents (dichloromethane: CH_2Cl_2; chloroform: $CHCl_3$; ethanol: EtOH; distilled water: H_2O; acetone: Me_2CO; and methanol: MeOH). PCAs show the first (PC1) and second (PC2) principal components.

that dichloromethane and chloroform had high total terpenoid content in *S. buxifolia* branches (0.74% and 0.46% w/w, respectively). By contrast, the lowest phenolic, flavonoid, and alkaloid content in the crude extracts were shown by these solvents. Acetone also effectively extracted phenolics (2.65 mg GAE/g DW), flavonoids (0.72 mg QE/g DW), alkaloids (0.88 mg AE/g DW), and terpenoids (0.47%, w/w).

3.3. Impact of Extraction Solvents on Antioxidant and In Vitro Anti-Inflammatory Capacities of S. buxifolia. In order to determine the influence of different solvents on the antioxidant and *in vitro* anti-inflammatory capacities of *S. buxifolia* branches, four inhibition assays (i.e., DPPH scavenging activity, albumin denaturation, proteinase inhibition, and membrane stabilization) of crude extracts were measured and indexed in IC_{50} values. A PCA and ANOVA models were conducted using the IC_{50} value of individual biological assay. The results indicated that the used solvents significantly affected the antioxidant and *in vitro* anti-inflammatory capacities of *S. buxifolia* branches ($p < 0.001$; Figure 3; Tables 2 and 3). As shown in Figure 3, the first two components accounted 90.5% of the observed variation (PC1 78.5% and PC2 12.0%). Most of tested biological activity assays revealed that the 50% inhibitory concentration of *S. buxifolia* crude extracts is in increasing order with the corresponding solvents used: $H_2O < CHCl_3 < CH_2Cl_2 < Me_2CO < EtOH < MeOH$, except in protease inhibition activity. Methanol and EtOH were highly separated in PCA results (Figure 3).

3.3.1. Antioxidant Activity of S. buxifolia Extracts. The antioxidant activities of different *S. buxifolia* extracts were indexed by the DPPH radical scavenging activity. As illustrated in Table 2, different extracts possessed varying free-radical scavenging activities ($p < 0.001$). Among the extracts tested, methanolic extract was the most potent extract with an IC_{50} value of 16.99 μg/mL. Compared to methanolic extract, although ethanolic extract and chloroform extract exhibited a lower radical scavenging activity with respective IC_{50} values of 27.08 μg/mL and 33.44 μg/mL, those extracts had significantly higher radical scavenging activity than ascorbic acid ($IC_{50} = 50.94$ μg/mL) (Table 2).

3.3.2. Anti-Inflammatory Activity

(1) Inhibition of Albumin Denaturation. Protein denaturation is a process by which proteins lose their structure due to the presence of other compounds, external stress, or heat, thus leading them to lose their biological functionality. Therefore, denaturation of tissue proteins is recognized as a marker of inflammation [22]. In this study, the *in vitro* anti-inflammatory activity of *S. buxifolia* extracts was measured for inhibitory activity against protein denaturation. Table 3 presents the inhibitory effect of different *S. buxifolia* extracts on protein denaturation. As shown in Table 3, the highest inhibitory activity against albumin denaturation was observed in the methanolic extract ($IC_{50} = 28.86$ μg/mL), followed by ethanolic extract and acetone extract with IC_{50} values of 35 μg/mL and 91.6 μg/mL, respectively. These extracts displayed significantly higher protein protection than aspirin, a standard anti-inflammatory drug, since aspirin only inhibited 33.61% of albumin denaturation at a concentration of 100 μg/mL. However, dichloromethane, chloroform, and water extracts exhibited a low ability to inhibit albumin denaturation with IC_{50} values of 592.00 μg/mL, 623.08 μg/mL, and 769.67 μg/mL, respectively.

Table 1: Effect of different solvents on phenolic, flavonoid, and alkaloid content of *S. buxifolia* branches.

Extraction solvent	Phenolics (mg GAE/g DW)	Flavonoids (mg QE/g DW)	Alkaloids (mg AE/g DW)	Terpenoids (%, g/g)
Distilled water	5.95 ± 0.10^{b}	0.63 ± 0.05^{c}	0.16 ± 0.01^{e}	0.43 ± 0.03^{e}
Ethanol	3.60 ± 0.03^{c}	0.69 ± 0.01^{bc}	1.34 ± 0.03^{b}	0.97 ± 0.08^{b}
Methanol	13.36 ± 0.34^{a}	1.92 ± 0.09^{a}	1.40 ± 0.03^{a}	1.25 ± 0.16^{a}
Chloroform	1.22 ± 0.05^{e}	0.66 ± 0.01^{bc}	0.19 ± 0.003^{e}	0.74 ± 0.06^{c}
Dichloromethane	0.63 ± 0.01^{f}	0.33 ± 0.01^{d}	0.62 ± 0.03^{d}	0.66 ± 0.05^{cd}
Acetone	2.65 ± 0.02^{d}	0.72 ± 0.01^{b}	0.88 ± 0.03^{c}	0.47 ± 0.04^{de}

All values are the mean ± SD ($n = 3$). Means within a column with different letters significantly differ by Tukey's test at $p < 0.05$.

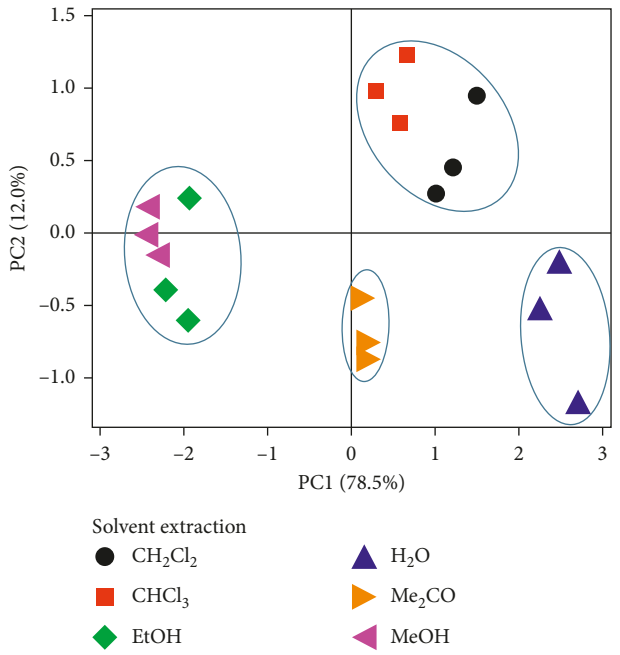

Figure 3: Principal component analysis of the antioxidant and *in vitro* anti-inflammatory capacities of *S. buxifolia* extracts by different solvents (dichloromethane: CH_2Cl_2; chloroform: $CHCl_3$; ethanol: EtOH; distilled water: H_2O; acetone: Me_2CO; and methanol: MeOH). PCAs show the first (PC1) and second (PC2) principal components.

Table 2: The 50% inhibitory concentration (IC_{50}) values of DPPH scavenging activity of different *S. buxifolia* extracts.

Samples	IC_{50} value (μg/mL)
Water extract	94.53 ± 9.51^{a}
Ethanolic extract	27.08 ± 6.61^{de}
Methanolic extract	16.99 ± 3.28^{e}
Chloroform extract	33.44 ± 5.39^{d}
Dichloromethane extract	71.87 ± 5.20^{b}
Acetone extract	66.07 ± 9.71^{b}
Ascorbic acid (control)	50.94 ± 2.72^{c}

All values are the mean ± SD ($n = 3$). Means within a column with different letters significantly differ by Tukey's test at $p < 0.05$.

(2) Antiproteinase Activity. Proteinases are involved in inflammatory reactions since leukocyte proteinases play a crucial role in tissue damage development during inflammatory reactions. As a result, proteinase inhibitors can provide a significant level of protection. In this study, the anti-inflammatory activity of *S. buxifolia* extracts was measured as indexed by antiproteinase activity. As presented in Table 3, the highest antiproteinase activity was obtained in methanolic extracts with IC_{50} of 414.29 μg/mL, followed by ethanolic extract ($IC_{50} = 418.80$ μg/mL), acetone extract ($IC_{50} = 575.5$ μg/mL), water extract ($IC_{50} = 601.8$ μg/mL), chloroform extract ($IC_{50} = 611.75$ μg/mL), and dichloromethane extract ($IC_{50} = 672.25$ μg/mL). However, all these extracts demonstrated a lower inhibitory activity against proteinase than aspirin, which inhibited 34.17% of proteinases at a concentration of 100 μg/mL.

(3) Membrane Stabilization. In this study, the effect of *S. buxifolia* extracts on membrane stabilization was indexed via the ability to protect RBCs from heat-induced haemolysis. As shown in Table 3, methanolic extract showed the highest inhibition against heat-induced haemolysis with IC_{50} of 319 μg/mL. In addition, ethanolic extract effectively protected the membrane against haemolysis induced by heat. However, low protective activity was observed in other extracts. As compared to *S. buxifolia* extracts, aspirin showed 29.26% protection at a concentration of 100 μg/mL. These results provide evidence for the membrane stabilizing effect of *S. buxifolia* extracts as an additional mechanism for their anti-inflammatory activity.

3.4. HPLC Analysis of the Enriched Extract of S. buxifolia Branches. HPLC analysis was carried out using the enriched extract of *S. buxifolia* branches, particularly in methanolic extract. 9(10H)-Acridanone was applied as the reference standard in this study. From the HPLC result (Figure 4), this compound was found in the methanolic extract of *S. buxifolia* branches (0.016% w/w; retention time: 5.108 min). Besides, some other peaks were also detected in the extract, which can be further isolated for identification as well as for investigating their properties.

4. Discussion

The use of bioactive compounds from natural sources as functional foods to promote human health and treat various diseases has been increasingly attracting considerable attention. In the present study, *S. buxifolia* was used as a natural source of secondary metabolite compounds such as phenolics, alkaloids, flavonoids, and terpenoids. To obtain bioactive compounds from the plant, there are several steps including grinding, milling, homogenization, and extraction [4]. Among these steps, extraction is the important step to recover and isolate bioactive compounds from the materials.

Table 3: *In vitro* anti-inflammatory activity of different *S. buxifolia* extracts.

Samples	IC_{50} values (μg/mL)		
	Albumin denaturation	Proteinase inhibition	Membrane stabilization
Water extract	769.67 ± 36.45^{a}	601.80 ± 36.43^{b}	829.00 ± 76.25^{a}
Ethanolic extract	35.00 ± 5.14^{d}	418.80 ± 51.94^{c}	356.71 ± 41.15^{c}
Methanolic extract	28.86 ± 4.80^{d}	414.29 ± 12.62^{c}	319.00 ± 20.43^{c}
Chloroform extract	623.08 ± 9.67^{b}	611.75 ± 29.67^{ab}	573.00 ± 13.76^{b}
Dichloromethane extract	592.00 ± 87.17^{b}	672.25 ± 57.32^{a}	525.00 ± 22.93^{b}
Acetone extract	91.60 ± 4.79^{c}	575.50 ± 15.57^{b}	596.17 ± 26.87^{b}
		Inhibitory activity, %	
Aspirin (100 μg/mL)	33.61 ± 0.56	34.17 ± 0.51	29.26 ± 1.87

All values are the mean ± SD ($n = 3$). Means within a column with different letters significantly differ by Tukey's test at $p < 0.05$.

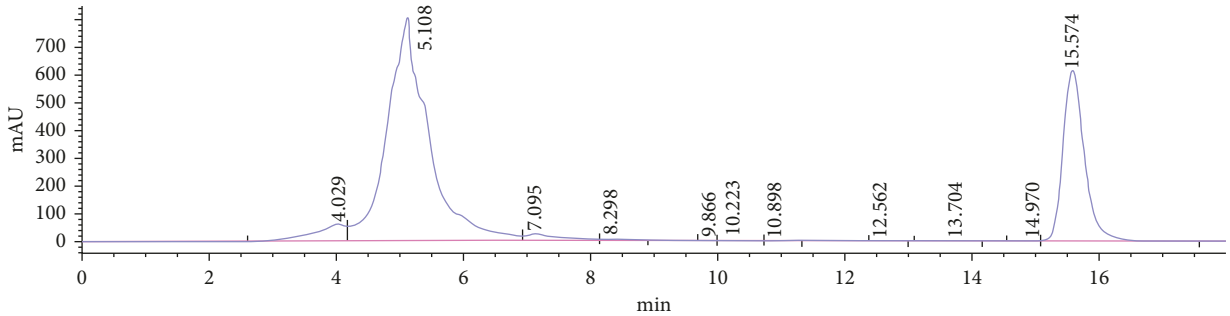

Figure 4: HPLC chromatogram detected at 250 nm for the methanolic extract of *S. buxifolia* branches.

Efficiency of the extraction is strongly affected by the extraction method, temperature, extraction time, the composition of phytochemicals, and the solvent used [13, 14, 23]. According to the results of these authors, under the same extraction conditions, solvent is recognized as one of the most important parameters. The present study used distilled water and organic solvents (methanol, ethanol, chloroform, dichloromethane, and acetone) to extract bioactive compounds from *S. buxifolia* branches. Results showed that different solvents resulted in various extraction yields. This is because differences in the polarity of the extraction solvents could cause a wide variation in the level of bioactive compounds in the extract. A higher extraction yield was observed in methanolic extract, distilled water extract, and ethanolic extract compared to chloroform, dichloromethane, and acetone extracts, indicating that the extraction efficiency favors the highly polar solvents. This result is consistent with the extraction yield of *Limophila aromatica* [4] and some other medicinal plants [24]. This could be because the plant material contains high levels of polar compounds that are soluble in solvents with high polarity such as water, methanol, and ethanol. In order to better understand the solvents effect on extraction yield, further analysis was performed to measure the content of bioactive compounds in the extract. In accordance with the extraction yields, the content of bioactive compounds (phenolics, alkaloids, flavonoids, and terpenoids) varied amongst the extracts. The highest levels of phenolics, flavonoids, alkaloids, and terpenoids were observed in methanolic extracts, thus resulting in the highest extraction yield of methanolic extract. This can be attributable to higher solubility of these compounds in methanol than the other solvents tested [4]. Taken together, these findings suggest that methanol is the best solvent for extracting bioactive compounds from *S. buxifolia* branches.

Extraction solvents have an effect on the extraction yield and the content of bioactive compounds, thus significantly affecting the biological activity of the extract [13, 14, 23]. In this study, the extracts obtained from different solvents were studied for their antioxidant activity by using DPPH scavenging activity assays. Among the extracts tested, the methanolic extract was the most potent in terms of IC_{50} values of DPPH scavenging activity. This could be because this extract contained the highest level of phenolic, flavonoid, alkaloid, and terpenoid compounds [22, 24, 25]. Those compounds possess powerful antioxidant activity and consequently protect the human body against oxidative damage through scavenging diverse reactive oxygen species, including hydroxyl radicals, peroxyl radicals, hypochlorous acid, peroxynitrite, and superoxide anions [25]. Remarkably, methanolic extract of *S. buxifolia* exhibited a three-fold higher DPPH scavenging activity than that of ascorbic acid. These findings suggest that the methanolic extract of *S. buxifolia* is a potential antioxidant agent for further drug development.

Inflammation is a complex process, associated with the reaction of body tissues to infection, irritation, or other injuries. Inflammation is therefore involved in various diseases such as asthma, diabetes, cancer, arthritis, neurodegenerative disorders, and autoimmune diseases [26]. The conventional treatment of inflammatory diseases by using steroidal and nonsteroidal anti-inflammatory drugs causes

serious side effects. Therefore, efforts have been made in recent years to search for new anti-inflammatory agents from natural sources because they are generally believed to be safer and more tolerable than conventional drugs to treat inflammation [6–8]. In the present study, the *in vitro* anti-inflammatory activity of *S. buxifolia* extracts were evaluated, indexed via the protective activity of the extract against albumin denaturation since tissue protein denaturation is one of the main causes of inflammation. Results showed that methanolic extract, ethanolic extract, and acetone extract of *S. buxifolia* effectively inhibited albumin denaturation. In addition, these extracts were found to have significantly higher protein protective capabilities than aspirin, indicating that *S. buxifolia* extracts have potential as novel anti-inflammatory agents.

The anti-inflammatory activities of *S. buxifolia* extracts were also indexed via antiproteinase activity since proteinases are involved in inflammation. Results showed that the *S. buxifolia* extracts demonstrated potential inhibitory activity against proteinase. In addition, RBC membrane stabilization was studied as a further index of the anti-inflammatory activity of *S. buxifolia* extracts since the RBC membrane has a similar structure to the lysosomal membrane. The inflammatory process lyses lysosome and results in the release of compounds that further propagate inflammation and consequently produce various disorders. Stabilization of membrane is a process of maintaining the integrity of the lysosomal membrane against heat-induced haemolysis, which prevents the release of fluids and serum proteins into the tissues caused by inflammatory mediators, thus inhibiting the inflammatory response [26]. In this study, methanolic extract of *S. buxifolia* effectively protected the RBC membrane against heat-induced haemolysis, indicating that the extract could well stabilize lysosomal membranes. It is possible that bioactive compounds in the extract protect lysosomal membranes against injury by interfering with activation of phospholipases. The results provide evidence for the anti-inflammatory activity of *S. buxifolia* extracts via a membrane stabilization effect.

The anti-inflammatory activities of the methanolic extract may be due to strong concentrations of phenolics, flavonoids, alkaloids, and terpenoids in the extract. Studies have demonstrated that these compounds possess powerful anti-inflammatory activity [27]. These findings suggest that methanolic extract of *S. buxifolia* is the most potent extract, and this is a promising source of anti-inflammatory and antioxidant agents.

5. Conclusion

The present study reports the extraction of *S. buxifolia* branches using different solvents. Among the solvents tested, methanol was the best solvent for extracting bioactive compounds from *S. buxifolia* since it resulted in the highest extraction yield and highest content of phenolics, alkaloids, flavonoids, and terpenoids. The antioxidant and *in vitro* anti-inflammatory activities of the extracts were also investigated. Compared with other extracts, methanolic extract of *S. buxifolia* exhibited the highest antioxidant and *in vitro* anti-inflammatory activity. These results suggest that methanol is the best solvent for bioactive compounds extraction from *S. buxifolia* branches and that methanolic extract is a promising antioxidant and anti-inflammatory agent for the nutraceutical and pharmaceutical industries.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

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