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Article

Effect of Extraction Solvents and Drying Methods on the Physicochemical and Antioxidant Properties of *Helicteres hirsuta* Lour. Leaves

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Abstract: *Helicteres hirsuta* Lour. (*H. hirsuta* L.) is widely distributed in southeast Asian countries and has been used traditionally as a medicinal plant. However, optimal conditions for preparation of dried materials for further processing and suitable solvents for the extraction of bioactive compounds have not been investigated. The objective of this study was to evaluate the effects of different extraction solvents and different drying conditions on the physicochemical properties and antioxidant capacity of the *H. hirsuta* L. leaves. The results showed that both extraction solvents and drying conditions had a significant impact on physicochemical and antioxidant properties of *H. hirsuta* L. leaves. Among the five solvents investigated, water could extract the highest level of solid content and phenolic compounds, whereas methanol was more effective for obtaining flavonoids and saponins than other solvents. The leaves dried under either hot-air drying at 80 °C (HAD80), or vacuum drying at 50 °C (VD50) yielded the highest amount of total phenolic compounds

(7.77 and 8.33 mg GAE/g, respectively) and total flavonoid content (5.79 and 4.62 mg CE/g, respectively), and possessed the strongest antioxidant power, while leaves dried using infrared drying at 30 °C had the lowest levels of bioactive compounds. Phenolic compounds including flavonoids had a strong correlation with antioxidant capacity. Therefore, HAD80 and VD50 are recommended for the preparation of dried *H. hirsuta* L. leaves. Water and methanol are suggested solvents to be used for extraction of phenolic compounds and saponins from *H. hirsuta* L. leaves for the potential application in the nutraceutical and pharmaceutical industries.

Keywords: *Helicteres hirsuta* Lour.; drying method; solvent; bioactive compounds; antioxidant

1. Introduction

Helicteres hirsuta Lour. (*H. hirsuta* L.) is a member of *Helicteres* genus of the plant family *Steculiaceae* [1,2]. *H. hirsuta* L. is widely distributed in southeast Asian countries, such as Vietnam, Cambodia, Laos, Indonesia, and Thailand, and it has been used as a traditional medicine for treatment of different ailments [2]. A recent study isolated the lignans, (±)-pinoresinol, (−)-boehmenan and (−)-boehmenan H from the stem of *H. hirsuta* and found that these compounds possessed cytotoxic activity against human lung carcinoma, hormone-dependent human prostate carcinoma and human breast carcinoma [1]. Therefore, *H. hirsuta* L. is a potential starting material for the extraction and isolation of bioactive compounds for further utilization in the nutraceutical and pharmaceutical industries.

Drying is an important process of dried material preparation for further processing because it reduces the moisture content of fresh materials for long storage and minimizes the costs of transportation and preservation. However, drying conditions have been shown to have significant influences on sensory quality, stability of bioactive compounds, and their antioxidant capacity [3–7]. Importantly, there is no previous study reporting the optimal drying conditions for *H. hirsuta* L. leaves. Therefore, it is necessary to identify the optimal drying conditions for preparation of dried *H. hirsuta* L. leaves for further processing steps.

Determination of optimal solvent for extraction of bioactive compounds from natural materials is also important for maximizing the extraction yield of bioactive compounds because these compounds have different characteristics as well as polarities [8]. Previous studies revealed that different contents of bioactive compounds and different antioxidant power were observed when extraction was conducted using different solvents [9–11]. Therefore, it is also necessary to determine the impact of solvents on extraction of bioactive compounds and antioxidant properties of *H. hirsuta* L. leaves to identify the most suitable solvent for extraction of bioactive compounds from *H. hirsuta* L. leaves. However, there is limited information in the literature.

This study aimed to determine the effect of five various extraction solvents (water, acetonitrile, ethanol, methanol and ethyl acetate) and four different drying methods (hot-air drying, low-temperature-air drying, infrared drying and vacuum drying) on physical, chemical and antioxidant properties of *H. hirsuta* L. leaves. The most suitable solvent and optimal drying conditions

drawn from this study could be used for further processing steps to prepare bioactive compounds from *H. hirsuta* L. leaves for utilization in the nutraceutical and pharmaceutical industries.

2. Experimental Section

2.1. Plant Material

Helicteres hirsuta Lour. leaves were collected from Hon Nghe village, Vinh Ngoc commune, Nha Trang city, Khanh Hoa province, Vietnam (latitude of 12.28°N, longtitude of 109.18°E) in November 2014. The leaves were cooled using plastic ice bags and immediately transported to the laboratory at Nha Trang University and stored in polyamide (PA) bags at −20 °C to minimize degradation of bioactive compounds and antioxidant property before being dried to constant weight.

2.2. Analytical Chemicals

Chemicals used in analytical experiments were as follows: Acetonitrile, ethanol, methanol, ethyl acetate, vanillin, sulphuric acid, and potassium persulfate were products of Merck (Darmstadt, Germany). Folin-ciocalteu's phenol regent, anhydrous sodium carbonate, sodium nitrile, ferric chloride, gallic acid, catechin, 2,4,6-Tris(2-pyridyl)-*s*-triazine; (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), escin, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich Co. (New South Wales, Australia). Sodium acetate trihydrate was purchased from Government Stores Department (New South Wales, Australia). Aluminum chloride was obtained from J. T. Baker Chem. Co. (Phillipsburg, NJ, USA). Acetic acid was obtained from BDH Laboratory Supplies (Poole, England). Sodium hydroxide was purchased from Ajax Chem. (New South Wales, Australia) and hydrochloride acid was obtained from Lab-scan Ltd. (Bangkok, Thailand).

2.3. Preparation of Dried H. hirsuta *L. Samples*

Frozen *H. hirsuta* L. leaves were thawed and dried to constant weight using four different drying methods (approximately 150 gram of sample for each drying condition), as described by Nguyen *et al.* [5] with some modifications.

Hot-air drying: Raw *H. hirsuta* L. leaves were dried in a hot-air oven (Ketong, Jiangsu, China) at two temperatures: 80 °C (HAD80) and 90 °C (HAD90).

Low-temperature-air drying: Drying was implemented in a low-temperature-air drying cabinet (SL-N, Nha Trang University, Vietnam) at two different temperatures: 30 °C (LTD30) and 35 °C (LTD35).

Infrared drying: Raw *H. hirsuta* L. leaves were dried with two infrared bulbs (250W/bulb) (Osram AG, Augsburg, Germany) in an infrared drying cabinet (SHN-L, Nha Trang University, Vietnam) at three temperatures: 30 °C (IRD30), 35 °C (IRD35) and 40 °C (IRD40).

Vacuum drying: Drying was performed in a vacuum oven (Memmert, Schwabach, Germany) at 50 °C and vacuum pressure of 65 mb (VD50).

After being dried to constant weight, *H. hirsuta* L. leaves were packed in PA bags using a vacuum packaging machine (Vacuum Gauge, Sun Jau Machine Industry Co., Ltd., Taiwan) with the following

parameters: Vacuum time of 40 s, diluting time of 4 s, seal time of 6 s, cooling time of 3 s and stored at −20 °C until required.

2.4. Experimental Design

Impact of solvent type: To determine the influence of extraction solvents on chemical and antioxidant properties of *H. hirsuta* L. leaves, infrared dried samples at 30 °C were ground and sieved to obtain the particle size <1.40 mm. These samples were then extracted using five different solvents of varying polarities (water, acetonitrile, ethanol, methanol, and ethyl acetate), with the ratio of solvent to dried samples at 100:1 mL/g. The mixtures were left to stand at room temperature (RT: 20 ± 1 °C) for twenty minutes of pre-leaching, then placed into an ultrasonic bath (Soniclean 220 V, 50 Hz, 250 W, Soniclean Pty Ltd., Thebarton, Australia) under following conditions: temperature (40 °C), time (60 min), power of 80% (200 W). Following ultrasonic extraction, the mixtures were cooled using an ice water bath to room temperature and filtered using Whatman no. 1 filter paper to collect the extracts for subsequent determination of bioactive compounds and antioxidant properties. The most suitable solvents were identified for the next experiment.

Impact of drying method: To evaluate the impact of different drying methods on bioactive compound yield and antioxidant activity of the leaves, the samples dried using the methods described above: hot-air drying, low-temperature-air drying, infrared drying and vacuum drying. Samples were then extracted in both water and methanol, the best solvents for phenolic compounds and saponins under similar extraction conditions as described above.

2.5. Determination of Physical Properties

The moisture content of the dried samples was determined according to the AOAC official methods of analysis (19th edition, 2012) [12] using the vacuum drying method at 95 °C for 24 h, and vacuum pressure of 60 kPa in a vacuum oven (Thermoline, NSW, Australia) to constant weight. The water activity of dried leaves was measured at 22 ± 1 °C using water activity meter (Pa_wkit, Decagon Devices, Washington, DC, USA).

The color of the dried samples was determined using colorimeter (Konica Minolta CR-400/410, Osaka, Japan). The *L*-, *a*- and *b*-values of the dried samples were recorded. The *L*-value is from 0 to 100 that expresses darkness to lightness; the positive or negative a-value relates to the redness or greenness of the sample; while the *b*-value shows the color range from yellow (positive *b*-value) to blue (negative *b*-value). Chroma, hue angle and browning index were calculated according to the formulas, as stated by Oliveira *et al.* [13].

2.6. Determination of Extractable Solid Content

Extractable solid content (ESC) was calculated by the weight of the dried extract per 100 g of dried sample (dried mass without water). ESC was determined by drying 3 mL of extract at 95 °C for 24 h, and pressure of 60 kPa in the vacuum oven (Thermoline, NSW, Australia) to constant weight. ESC was expressed as g dried extract per 100 g dried sample.

2.7. Determination of Bioactive Compounds and Antioxidant Capacity of H. hirsuta *L. Leaves*

Each of the test results was determined on the basis of dry plant mass (water excluded) normalized using moisture content data from Table 4.

2.7.1. Bioactive Compounds

Total Phenolic Content (TPC): TPC of the extracts was measured as described by Vuong *et al.* [11] with some modifications. To 0.5 mL of extract was added 2.5 mL of 10% (*v*/*v*) Folin-Ciocalteu reagent and left at RT for 8 min, then mixed with 2 mL of 7.5% (*w*/*v*) Na_2CO_3. The mixture was incubated in the dark at RT for 1 h, then its absorbance was measured at 765 nm using a UV-VIS spectrophotometer (Cary 50 Bio, Varian Australia Pty. Ltd., Victoria, Australia). Gallic acid with the concentration range from 10 µg/mL to 100 µg/mL, was used for the standard curve and the total phenolic content was expressed as mg gallic acid equivalents per gram of dried sample (mg GAE/g).

Total Flavonoid Content (TFC): TFC of the extracts was determined as described by Vuong *et al.* [11]. To 0.5 mL of extract was added 2 mL of deionized (DI) water and 0.15 mL of 5% (*w*/*v*) $NaNO_2$, and the mixture was left at RT for 6 min. Then, 0.15 mL of 10% (*w*/*v*) of $AlCl_3$ was added and allowed to stand for 6 min, followed by the addition of 2 mL of 4% (*w*/*v*) NaOH and 0.7 mL of DI water. The mixture was mixed thoroughly and left at RT for 15 min. The absorbance was measured at 510 nm using a UV-VIS spectrophotometer (Cary 50 Bio, Varian Australia Pty. Ltd., Victoria, Australia). Catechin with the concentration range from 10 µg/mL to 100 µg/mL was used as a standard and the total flavonoid content was expressed as mg catechin equivalents per gram of dried sample (mg CE/g).

Saponin Content: The content of saponins was measured as described by Vuong *et al.* [11] with some modifications. Briefly, 0.25 mL of the extract was mixed with 0.25 mL of 8% (*w*/*v*) vanillin, followed by the addition of 2.5 mL of 72% (*v*/*v*) H_2SO_4. The mixture was incubated in a water bath (Ratek, Ratek Instruments Pty. Ltd., Victoria, Australia) at 60 °C for 15 min, then cooled using an ice water bath to RT and the absorbance measured at 560 nm using a UV-VIS spectrophotometer (Cary 50 Bio, Varian Australia Pty. Ltd., Victoria, Australia). A concentration series of standard escin from 100 µg/mL to 1000 µg/mL were prepared to generate the standard curve and the content of saponins was expressed as mg escin equivalents per gram of dried sample (mg ESE/g).

2.7.2. Antioxidant Capacity

DPPH Radical Scavenging Capacity: DPPH radical scavenging ability was assessed as described by Vuong *et al.* [11] with some modifications. Firstly, 24 mg of DPPH was dissolved in 100 mL methanol to obtain a stock solution that was kept in the dark at −20 °C for further use. Then, 10 mL of stock solution was added to 45 mL methanol to provide the working solution which its absorbance value was 1.1 ± 0.02 at 515 nm. The sample (0.15 mL) was added to 2.85 mL of the working solution and left in the dark at RT for 3 h, then its absorbance was measured at 515 nm using a UV-VIS spectrophotometer (Cary 50 Bio, Varian Australia Pty. Ltd., Victoria, Australia). Trolox with the concentration range from 12.5 µM to 800 µM was used as a standard and the results were expressed as mg trolox equivalents per gram of dried sample (mg TE/g).

ABTS Radical Scavenging Capacity: ABTS radical scavenging activity was determined based on the methods described by Thaipong *et al.* [14] and Kamonwannasit *et al.* [15] with few modifications. A stock solution was prepared by adding 10 mL of 7.4 mM ABTS solution in 10 mL of 2.6 mM $K_2S_2O_8$ and left at RT in the dark for 15 h, and then stored at −20 °C until required. The working solution was freshly prepared by diluting 1 mL of stock solution with 60 mL of methanol to obtain an absorbance value of 1.1 ± 0.02 at 734 nm. To 0.15 mL of sample was mixed 2.85 mL of the working solution and left in the dark at RT for 2 h, and the absorbance was then read at 734 nm using a UV-VIS spectrophotometer (Cary 50 Bio, Varian Australia Pty. Ltd., Victoria, Australia). Trolox with the concentration range from 25 μM to 500 μM was used as a standard and the results were expressed as mg trolox equivalents per gram of dried sample (mg TE/g).

Ferric Reducing Antioxidant Power (FRAP): FRAP was measured as described by Thaipong *et al.* [14] and Kamonwannasit *et al.* [15] with some modifications. A working FRAP solution was prepared by mixing 300 mM acetate buffer, 10 mM TPTZ (2, 4, 6-tripyridyl-*s*-triazine) in 40 mM HCl and 20 mM $FeCl_3$ in the ratio of 10:1:1 and warmed at 37 °C in a water bath (Ratek, Ratek Instruments Pty. Ltd., Victoria, Australia) before used. The sample (0.15 mL) was added to 2.85 mL of the working FRAP solution and incubated at RT in the dark for 30 min before reading its absorbance at 593 nm. Trolox with the concentration range from 12.5 μM to 500 μM was used as a standard and the results were expressed as mg trolox equivalents per gram of dried sample (mg TE/g).

2.8. Statistical Analyses

Experiments were performed in triplicate, and means ±SD were assessed using one-way ANOVA and Duncan of *Post Hoc* Multiple Comparisons with IBM SPSS Statistics 22. Differences in means were considered statistically significant at $p < 0.05$.

3. Results and Discussion

3.1. Effect of Solvents on Extractable Solid Content, Bioactive Compounds and Antioxidant Capacity of H. hirsuta *L. Leaves*

3.1.1. Effect on Extractable Solid Content

The results (Figure 1) showed that extraction solvents significantly affected extractable solids of *H. hirsuta* L. leaves ($p < 0.05$). Water was demonstrated to be the most suitable solvent to obtain the highest amount of solids (12.10 g dried extract/100 g dried sample), followed by methanol (5.34 g dried extract/100 g dried sample), acetonitrile, ethanol, and ethyl acetate, which resulted in the lowest extractable solid contents (1.09, 1.94 and 1.81 g dried extract/100 g dried sample, respectively). A similar result was found by Nguyen *et al.* [16] that water and methanol extracts of *Paramignya trimera* had higher extraction yields than acetonitrile, ethyl acetate and hexane extracts. In addition, Jaiswl *et al.* [9] found that water was the best solvent for the highest extraction yield of Irish York cabbage in comparison with methanol, ethanol and acetone.

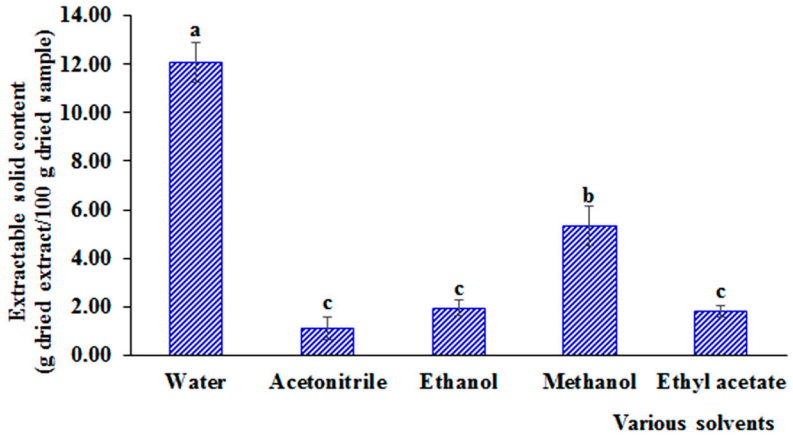

Figure 1. Effect of various solvents on extractable solid content. The bars are means ± standard deviations of triplicate experiments and those that have different letters (a–c) on top of the columns differ significantly ($p < 0.05$).

3.1.2. Effect on Bioactive Compounds

The results (Figure 2) indicated that extraction solvents also significantly affected extraction yields of polyphenols, flavonoids and saponins ($p < 0.05$); however, the effect of extraction on each type of bioactive compounds was different. Water was found to be the best solvent for extraction of phenolic compounds (3.94 mg GAE/g), followed by methanol (2.55 mg GAE/g); whereas, ethanol, acetonitrile and ethyl acetate were not suitable for extraction of phenolic compounds from *H. hirsuta* L. leaves (Figure 2a). The variation could be explained by the different polarity of the solvents and majority of the phenolic compounds in *H. hirsuta* L. leaves was hydrophilic. These results were similar to the findings of Vuong *et al.* [11] who found that water had greater extraction yield for polyphenols from papaya leaves than that of acetone, ethanol and methanol. However, these results were different to the findings of Rezaie *et al.* [17], who showed that ethanol and methanol were more effective for extraction of phenolic compounds from *Pistacia atlantica* subsp. *mutica* than water. This can be explained by the characteristics of phenolic compounds. Water was the best solvent in this study for phenolic compounds because phenolic compounds from *H. hirsuta* L. leaves were more hydrophilic. The results (Figure 2b,c) showed that methanol was the best solvent for extraction of flavonoids and saponins. Less than a third of flavonoids and saponins were extracted when using water, ethanol, acetonitrile and ethyl acetate as extraction solvents. From the results outlined above, it could be concluded that water was the best solvent for extraction of phenolic compounds from *H. hirsuta* L. leaves, whereas methanol was the solvent of choice for extraction of saponins and flavonoids.

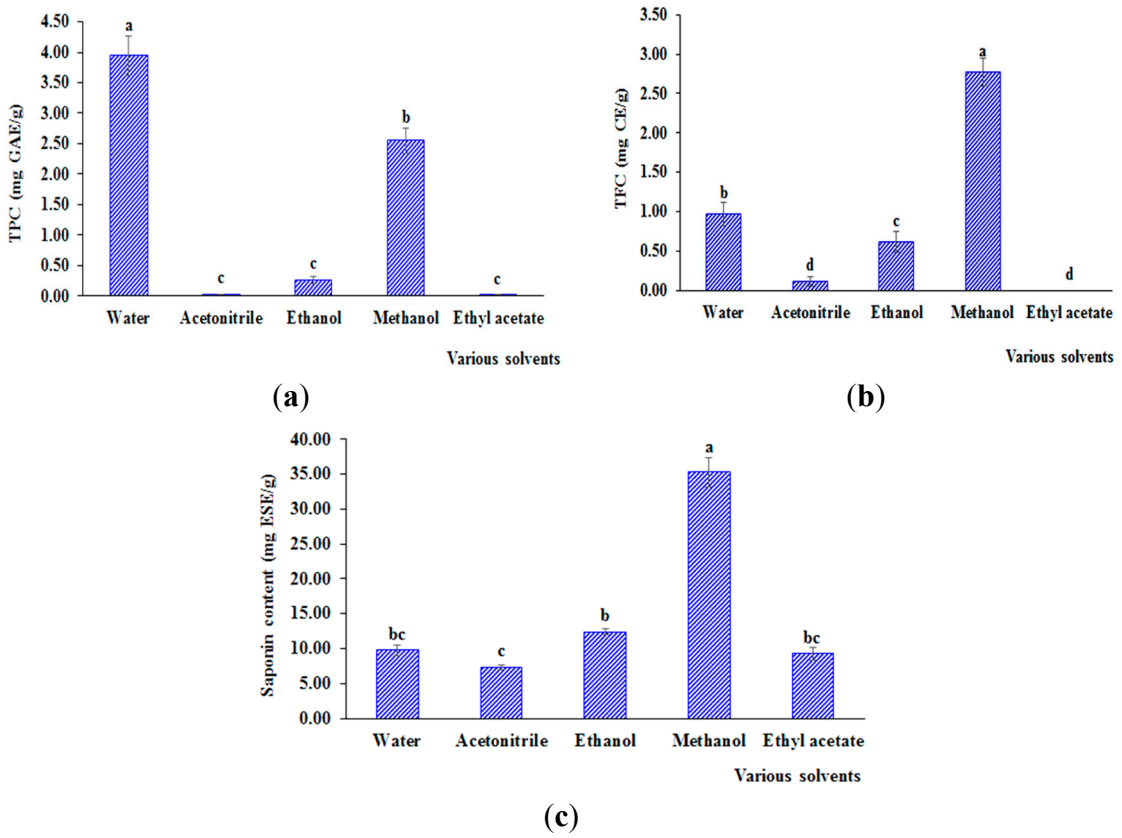

Figure 2. Effect of various solvents on bioactive compound yield of *H. hirsuta* L. leaves. (**a**) Total phenolic content (TPC); (**b**) Total flavonoid content (TFC); (**c**) Saponin content. GAE: Gallic acid equivalents; CE: Catechin equivalents; ESE: Escin equivalents. The bars are means ± standard deviations of triplicate experiments and those that have different letters (a–d) on top of the columns differ significantly ($p < 0.05$).

3.1.3. Effect on Antioxidant Capacity

Results from three antioxidant assays (Figure 3) revealed that extraction solvents also had a significant impact on antioxidant properties of *H. hirsuta* L. leaves ($p < 0.05$). Water and methanol extracts had greater antioxidant capacity than those prepared by acetonitrile, ethyl acetate and ethanol. Water extract possessed similar DPPH free radical scavenging capacity and ferric reducing power to those of methanol extract (Figure 3a,b). However, water extract had significantly greater ABTS radical scavenging capacity than that of methanol extract ($p < 0.05$; Figure 3c). These results had a strong link with those of extractable solid mass and the bioactive compound content identified above. It was observed that the greater the amount of bioactive compounds in the extract, the stronger antioxidant capacity was obtained. Therefore, water and methanol were chosen for further extraction processes to evaluate the effect of different drying methods on bioactive component yield and antioxidant capacity.

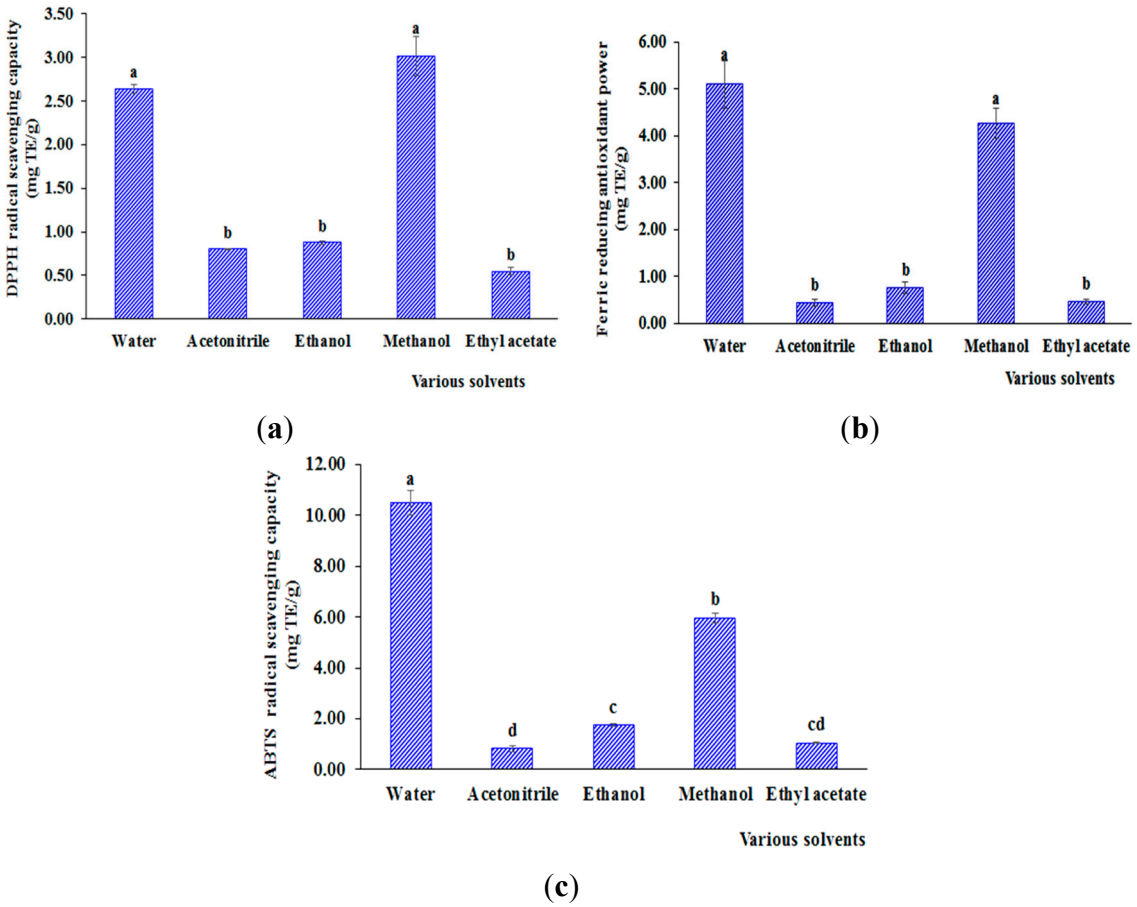

Figure 3. Effect of various solvents on antioxidant capacity of *H. hirsuta* L. leaves. (**a**) DPPH radical scavenging capacity; (**b**) Ferric reducing antioxidant power; (**c**) ABTS radical scavenging capacity. TE: Trolox equivalents. The bars are means ± standard deviations of triplicate experiments and those that have different letters (a–d) on top of the columns are significantly different ($p < 0.05$).

3.2. Impact of Drying Methods on Extractable Solid Content, Bioactive Compound Yield and Antioxidant Capacity of Aqueous and Methanol Extracts from H. hirsuta *L. Leaves*

3.2.1. Impact on Extractable Solid Content

The amount of extractable solids in the aqueous extracts was not significantly affected across the eight different drying conditions ($p > 0.05$; Figure 4), with the mean value of 11.58 g dried extract/100 g dried sample. However, various drying methods had a significant influence on solid content extracted in methanol ($p < 0.05$), with an observed range of 5.24 to 9.78 g dried extract/100 g dried sample. Specifically, the highest content of solids in the methanol extract was observed in the infrared dried sample at 35 °C. The difference between infrared drying and convective heating drying methods is that material directly absorbed infrared energy rather than requiring the transfer of heat from air. Infrared radiation penetrates and collides with the material that is converted to sensible heat [18]. Drying processing was implemented at different temperatures under or not under vacuum. These caused the changes of chemical components and physical properties of *H. hirsuta* L. leaves such as the structure of leaf tissues and solubility of solids in methanol, and consequently the amount of dissolvable solids in

methanol differed significantly among different drying conditions ($p < 0.05$). These findings were supported by the results shown in the study of Nguyen *et al.* [5] that the highest extraction yield in 70% ethanol of *Phylanthus amarus* was found in the sample dried using infrared drying method at 40 °C (17.17 g dried extract/100 g dried sample) in comparison with other drying methods.

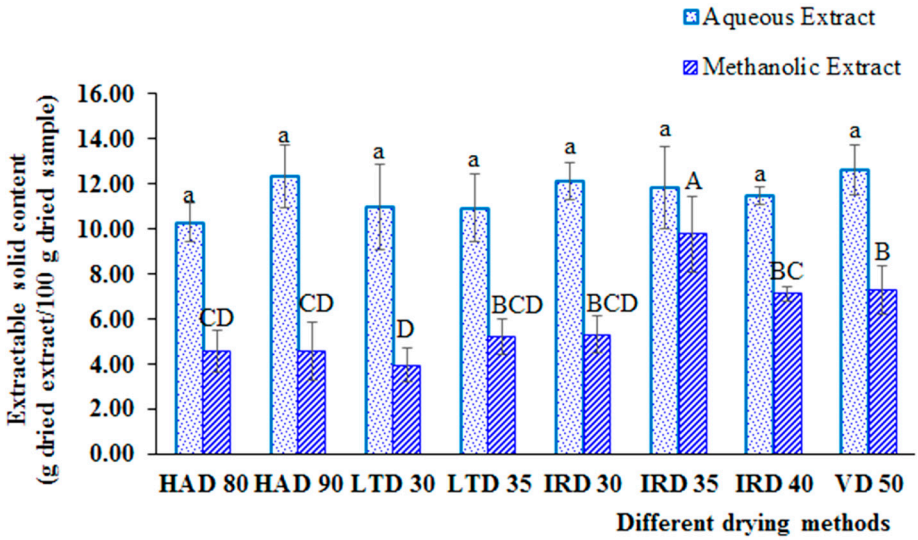

Figure 4. Effect of different drying methods on extractable solid content. HAD80/HAD90: Hot-air drying at 80 °C/90 °C; LTD30/LTD35: Low-temperature-air drying at 30 °C/35 °C; IRD30/IRD35/IRD40: Infrared drying at 30 °C/35 °C/40 °C; VD50: Vacuum drying at 50 °C. The bars are means ± standard deviations of triplicate experiments and those that have different letters (A–D) on top of the columns differ significantly ($p < 0.05$).

3.2.2. Impact on Bioactive Compounds

The results in Table 1 showed that different drying methods had a significant impact on levels of polyphenols, flavonoids and saponins remaining in the *H. hirsuta* L. dried leaves ($p < 0.05$). Dried *H. hirsuta* L. leaves prepared using hot-air drying at 80 °C (HAD80) and vacuum drying at 50 °C (VD50) contained the highest levels of phenolic compounds and flavonoids. In contrast, both infrared and low-temperature-air drying methods at 30 °C resulted in significantly lower levels of phenolic compounds and flavonoids ($p < 0.05$). For the same drying method, such as infrared drying and low-temperature-air drying, increasing the temperature from 30 to 35 °C (low-temperature-air drying) and from 30 to 40 °C (infrared drying), dried leaves had higher levels of phenolic compounds and flavonoids. However, in the hot-air drying method, increasing temperature from 80 to 90 °C resulted in a significant fall in phenolic compounds and flavonoids ($p < 0.05$). These could be explained by the fact that the drying time was extended at lower temperatures, such that the samples had a longer duration of oxygen exposure resulting in increased redox activity and degradation of phenolic compounds. For example, it took 18 h for the sample dried at IRD30 to obtain constant weight, whereas, only 6 h was recorded when IRD40 was applied (Table 4). In contrast, while the application of hot-air drying inactivated enzymes over a shorter duration, the higher temperature (90 °C) would significantly damage heat-sensitive bioactive components ($p < 0.05$).

Table 1. Impact of drying methods on total phenolic content (TPC) and total flavonoid content (TFC) of aqueous extract and methanol extract from *H. hirsuta* L. leaves.

Drying Method	TPC (mg GAE/g)		TFC (mg CE/g)	
	Aqueous Extract	Methanol Extract	Aqueous Extract	Methanol Extract
HAD80	7.77 ± 0.67 [a]	3.57 ± 0.59 [a]	5.79 ± 0.07 [a]	4.75 ± 0.36 [a]
HAD90	6.58 ± 0.59 [b]	2.34 ± 0.11 [bc]	3.25 ± 0.23 [c]	3.55 ± 0.26 [ab]
LTD30	4.52 ± 0.40 [ed]	1.91 ± 0.07 [c]	1.48 ± 0.09 [e]	2.40 ± 0.23 [b]
LTD35	5.70 ± 0.46 [bc]	2.75 ± 0.08 [b]	1.99 ± 0.02 [d]	4.58 ± 0.31 [a]
IRD30	3.99 ± 0.33 [f]	2.44 ± 0.17 [bc]	0.97 ± 0.15 [f]	2.86 ± 0.17 [b]
IRD35	4.41 ± 0.39 [ed]	2.39 ± 0. 21 [bc]	1.33 ± 0.01 [e]	3.47 ± 0.14 [ab]
IRD40	5.35 ± 0.24 [cd]	2.41 ± 0.15 [bc]	1.87 ± 0.06 [d]	3.38 ± 0.11 [ab]
VD50	8.33 ± 0.56 [a]	4.06 ± 0.11 [a]	4.62 ± 0.02 [b]	4.76 ± 1.45 [a]

GAE: Gallic acid equivalents; CE: Catechin equivalents. HAD80/HAD90: Hot-air drying at 80 °C/90 °C; LTD30/LTD35: Low-temperature-air drying at 30 °C/35 °C; IRD30/IRD35/IRD40: Infrared drying at 30 °C/35 °C/40 °C; VD50: Vacuum drying at 50°C. The values are means ± standard deviations of triplicate experiments and those in the same column that have different superscript letters ([a–f]) differ significantly ($p < 0.05$).

It was clear that various drying methods, conditions (temperature range 30–90 °C, and drying time range 2–19 h; Table 4) had a significant influence on phenolic compounds and flavonoids of *H. hirsuta* L. leaves ($p < 0.05$; Table 1). Our results showed that samples dried using hot-air drying at 80 °C and vacuum drying at 50 °C retained the greatest yield of TPC and TFC among the eight drying conditions assessed. Nguyen *et al.* [5] reported that drying *P. amarus* using infrared method at 30 °C could maintain polyphenols as well as flavonoids more effectively than other tested drying conditions. Different starting materials are likely the main reason for this inconsistency. Orphanides *et al.* [19] indicated that various drying treatments had a great influence on phenolic composition and antioxidant ability of dried spearmint, in which spearmint prepared using freeze drying had the highest content of phenolic compounds while convection oven drying and microwave drying methods caused a remarkable decrease of these compounds, approximately three times lower than freeze drying method.

Figure 5 illustrates that saponin levels were also significantly affected by various drying conditions ($p < 0.05$). VD50 and HAD90 were found to be more effective for retention of saponins than other drying methods. The VD50 and HAD90 methanol extract contained the highest saponin contents (49.06 mg ESE/g and 47.66 mg ESE/g, respectively), whereas, LTD30 and IRD30 had the lowest levels of saponins (34.46 and 34.49 mg ESE/g, respectively). These data revealed that different drying treatments resulted in a significant variation on the saponin content of *H. hirsuta* L. leaves ($p < 0.05$) and the drying methods that had the highest retention of saponins were VD50 and HAD90.

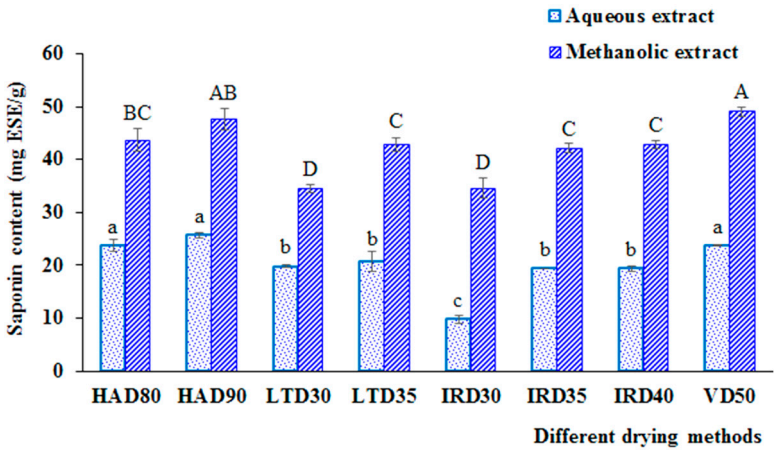

Figure 5. Impact of drying methods on saponin content of aqueous and methanol extract. ESE: Escin equivalents. HAD80/HAD90: Hot-air drying at 80 °C/90 °C; LTD30/LTD35: Low-temperature-air drying at 30 °C/35 °C; IRD30/IRD35/IRD40: Infrared drying at 30 °C/35 °C/40 °C; VD50: Vacuum drying at 50 °C. The bars are means ± standard deviations of triplicate experiments and those in the same column that have different letters on top of the columns (a–c or A–D) differ significantly ($p < 0.05$).

3.2.3. Impact on Antioxidant Capacity

Results from three antioxidant assays including DPPH radical scavenging capacity, ABTS radical scavenging capacity and FRAP revealed that different drying conditions and methods also significantly affected antioxidant capacity of dried *H. hirsuta* L. leaves ($p < 0.05$; Table 2).

Table 2. Impact of drying methods on antioxidant capacity of *H. hirsuta* L. leaves.

Drying Method	DPPH Radical Scavenging Capacity (mg TE/g)		ABTS Radical Scavenging Capacity (mg TE/g)		Ferric Reducing Antioxidant Power (mg TE/g)	
	Aqueous Extract	Methanol Extract	Aqueous Extract	Methanol Extract	Aqueous Extract	Methanol Extract
HAD80	14.23 ± 0.16 ^{a}	3.78 ± 0.22 ^{b}	18.14 ± 0.08 ^{a}	7.22 ± 0.36 ^{b}	15.09 ± 0.07 ^{a}	7.31 ± 0.40 ^{a}
HAD90	9.33 ± 0.57 ^{b}	3.28 ± 0.16 ^{bc}	12.83 ± 0.37 ^{c}	5.15 ±0.45 ^{cd}	8.41 ± 0.65 ^{c}	5.11 ± 0.36 ^{bcd}
LTD30	5.09 ± 0.38 ^{e}	3.35 ± 0.41 ^{bc}	9.19 ± 0.50 ^{f}	4.29 ±0.62 ^{d}	5.46 ± 0.39 ^{e}	3.93 ± 0.60 ^{e}
LTD35	7.76 ± 0.11 ^{c}	3.78 ± 0.17 ^{b}	12.64 ± 0.08 ^{c}	5.83 ± 0.61 ^{c}	7.22 ± 0.49 ^{d}	5.66 ± 0.41 ^{b}
IRD30	2.64 ± 0.05 ^{f}	3.01 ± 0.23 ^{c}	10.49 ± 0.48 ^{e}	5.96 ± 0.21 ^{c}	5.09 ± 0.51 ^{e}	4.27 ± 0.32 ^{de}
IRD35	4.67 ± 0.41 ^{e}	3.23 ± 0.28 ^{bc}	10.98 ± 0.44 ^{de}	4.08 ± 0.42 ^{d}	5.53 ± 0.46 ^{e}	4.47 ± 0.25 ^{cde}
IRD40	6.66 ± 0.49 ^{d}	3.14 ± 0.23 ^{bc}	12.08 ± 0.25 ^{cd}	5.76 ± 0.26 ^{c}	7.17 ± 0.37 ^{d}	5.39 ± 0.03 ^{bc}
VD50	13.45 ± 0.18 ^{a}	4.73 ± 0.16 ^{a}	16.77 ± 0.53 ^{b}	8.50 ± 0.75 ^{a}	13.54 ± 0.19 ^{b}	7.98 ± 0.59 ^{a}

TE: Trolox equivalents. HAD80/HAD90: Hot-air drying at 80 °C/90 °C; LTD30/LTD35: Low-temperature-air drying at 30 °C/35 °C; IRD30/IRD35/IRD40: Infrared drying at 30 °C/35 °C/40 °C; VD50: Vacuum drying at 50 °C. The values are means ± standard deviations of triplicate experiments and those in the same column that have different superscript letters ($^{a–f}$) are significantly different ($p < 0.05$).

The results demonstrated that increasing temperature within a range of 30 °C and 40 °C in a same drying method (low-temperature-air or infrared drying method) resulted in the rise of antioxidant ability. However, the data for DPPH, ABTS and FRAP analysis revealed that hot-air drying had a different

trend. Specifically, increasing temperature from 80 to 90 °C caused a sharp fall of FRAP. This is explained by the fact that high temperatures would destroy antioxidant components that contribute to the radical inhibitory activity. DPPH and ABTS radical scavenging ability of samples treated using HAD80 and VD50 were greater than HAD90, LTD35, LTD30, IRD30, IRD35 and IRD40. These results confirmed that HAD80 and VD50 were the most suitable drying conditions for obtaining dried *H. hirsuta* L. leaves with potent antioxidant capacity.

3.2.4. Correlation of Bioactive Compounds in Dried *H. hirsuta* L. Leaves and Antioxidant Capacity

Table 3 revealed that phenolic compounds and flavonoids had a strong positive correlation with antioxidant capacity. Saponins were found to have less correlation with antioxidant capacity in comparison with phenolic compounds and flavonoids. These findings revealed that phenolic compounds including flavonoids were the major contributors to antioxidant capacity of dried *H. hirsuta* L. leaves. These results were supported by the previous findings of Vuong *et al.* [11], in which phenolic compounds of the papaya leaf had a close relationship with total antioxidant capacity. In addition, Hung *et al.* [20] also showed that free phenolics in some certain vegetables correlated strongly with antioxidant activity.

Table 3. The correlation of bioactive compounds and antioxidant activity of the aqueous extract from *H. hirsuta* L. leaves.

Bioactive Compounds	*R*-Squared Value *		
	DPPH Radical Scavenging Capacity	ABTS Radical Scavenging Capactity	Ferric Reducing Antioxidant Power
TPC	0.97	0.95	0.92
TFC	0.96	0.96	0.97
Saponins	0.56	0.55	0.48

* *R*-squared value calculated from analysis of aqueous extract using different drying methods.

3.3. Impact of Drying Methods on the Physical Properties of H. hirsuta *L. Leaves*

Table 4 reveals that drying conditions also affected on moisture content, water activities and colour of dried *H. hirsuta* L. leaves. The moisture content of the dried samples using eight different drying conditions ranged from 3.09% to 8.67%, among them the HAD90 sample had the lowest moisture content, followed by HAD80 sample (5.51%). The explanation for this is that drying at higher temperatures (80 °C and 90 °C) stimulated water evaporation more vigorously and drying time was reduced as a consequence. For instance, drying time decreased by half when drying temperature was increased from 80 to 90 °C using the hot-air drying method. However, drying at lower temperatures such as LTD30, LTD35, IRD30, IRD35, IRD40 could not dehydrate the sample rapidly nor completely, especially the bound water which would result in higher residual moisture content, as well as a longer drying time.

Table 4. Impact of drying methods on drying time, moisture content and water activity of dried *H. hirsuta* L. leaves.

Drying Method	Drying Time (Hours)	Moisture Content (%)	Water Activity
HAD80	4.0	5.51 ± 0.53 ^{c}	0.49 ± 0.01 ^{e}
HAD90	2.0	3.09 ± 0.35 ^{d}	0.41 ± 0.01 ^{f}
LTD30	10.5	8.18 ± 0.44 ^{a}	0.59 ± 0.00 ^{c}
LTD35	9.0	6.93 ± 0.35 ^{b}	0.57 ± 0.01 ^{d}
IRD30	18.0	8.67 ± 0.15 ^{a}	0.68 ± 0.01 ^{a}
IRD35	12.0	8.35 ± 0.25 ^{a}	0.59 ± 0.00 ^{c}
IRD40	6.0	8.51 ± 0.35 ^{a}	0.61 ± 0.00 ^{b}
VD50	19.0	6.13 ± 0.58 ^{bc}	0.49 ± 0.01 ^{e}

HAD80/HAD90: Hot-air drying at 80 °C/90 °C; LTD30/LTD35: Low-temperature-air drying at 30 °C/35 °C; IRD30/IRD35/IRD40: Infrared drying at 30 °C/35 °C/40 °C; VD50: Vacuum drying at 50 °C. The values are means ± standard deviations of triplicate experiments and those in the same column that have different superscript letters ($^{a–f}$) differ significantly ($p < 0.05$).

The results showed that *H. hirsuta* L. leaves after being dried had water activity values ranging from 0.41 to 0.68, which could be stored for a long time with minimum degradation because Chirife *et al.* [21] reported that a_w of below 0.86 causes no proliferation of pathogen bacteria, and no yeasts and molds can develop at $a_w < 0.62$. Also, it is reported that most enzymatic reactions are slowed down at $a_w < 0.80$ [22].

Results (Table 5) showed that dried samples at low temperatures such as 30 °C and 35 °C had increased lightness and lower browning index values. For example, IRD30 sample possessed lightness of 10.70 and browning index of 42.58, whereas those of HAD80 sample were 8.88 and 51.99, respectively. These results indicated that low temperature drying procedures such as IRD30 and LTD35 maintained the lightness of dried *H. hirsuta* L. leaves, however they caused a sharp degradation of bioactive components. The decrease in the contents of bioactive components can be explained due to the fact that they were exposed to oxygen for longer time periods (Table 4). Table 5 also showed that Chroma and Hue angle values were also affected by different drying conditions. Chroma values accounted for a range between 3.39 and 4.36 while the Hue angle values ranged from 90.14 to 96.39. Chroma in the HAD90 sample was 4.36, followed by the VD50 sample of 4.24 and the LTD35 sample of 4.04, which were more intense than those of other drying conditions.

Table 5. Impact of drying methods on the color characteristic of *H. hirsuta* L. leaves.

Drying Method	Color Characteristic			
	Lightness	Chroma	Hue Angle (°)	Browning Index
HAD80	8.88 ± 0.06 ^{d}	3.78 ± 0.07 ^{e}	92.46 ± 0.80 ^{c}	51.99 ± 0.85 ^{a}
HAD90	10.39 ± 0.16 ^{b}	4.36 ± 0.03 ^{a}	93.94 ± 0.48 ^{b}	50.21 ± 1.32 ^{b}
LTD30	9.57 ± 0.18 ^{c}	3.39 ± 0.05 ^{g}	90.14 ± 1.13 ^{d}	42.44 ± 0.70 ^{e}
LTD35	10.83 ± 0.43 ^{a}	4.04 ± 0.09 ^{c}	93.67 ± 0.67 ^{b}	43.36 ± 2.02 ^{de}
IRD30	10.70 ± 0.27 ^{a}	3.86 ± 0.11 ^{d}	91.68 ± 0.78 ^{c}	42.58 ± 0.34 ^{e}

Table 5. *Cont.*

Drying Method	Color Characteristic			
	Lightness	**Chroma**	**Hue Angle (°)**	**Browning Index**
IRD35	9.75 ± 0.19 ^{c}	3.65 ± 0.02 ^{f}	90.58 ± 0.68 ^{d}	45.29 ± 1.05 ^{c}
IRD40	10.31 ± 0.21 ^{b}	3.79 ± 0.08 ^{de}	90.49 ± 1.03 ^{d}	44.27 ± 0.91 ^{cd}
VD50	10.87 ± 0.29 ^{a}	4.24 ± 0.07 ^{b}	96.39 ± 1.34 ^{a}	44.01 ± 1.11 ^{d}

HAD80/HAD90: Hot-air drying at 80 °C/90 °C; LTD30/LTD35: Low-temperature-air drying at 30 °C/35 °C; IRD30/IRD35/IRD40: Infrared drying at 30 °C/35 °C/40 °C; VD50: Vacuum drying at 50 °C. The values are means ± standard deviations of triplicate experiments and those in the same column that have different superscript letters ($^{a–g}$) differ significantly ($p < 0.05$).

4. Conclusions

The current study found that physical, chemical and antioxidant properties of *H. hirsuta* L. leaves were significantly affected by different solvents used for extraction and different drying methods applied to prepare dried leaves for further processing steps ($p < 0.05$). Our results demonstrated that water was the most suitable for extraction of phenolic compounds and methanol was the solvent of choice for extraction of saponins and flavonoids. Among the eight drying conditions assessed, both hot-air drying (80 °C) and vacuum drying (50 °C) were the most suitable drying conditions to prepare dried *H. hirsuta* L. leaves which had the highest levels of phenolic compounds and saponin content in comparison with other drying conditions. Of which, vacuum drying (50 °C) was the best drying method for saponin retention and color preservation (providing the highest Hue angle value), whereas infrared drying (30 °C) resulted in remarkable reduction of bioactive compound yield and antioxidant capacity of *H. hirsuta* L. leaf extracts. In addition, there were very strong positive correlations between total phenolic content, total flavonoid content, and their antioxidant capacity. The findings of this study can be utilized for preparation of dried *H. hirsuta* L. leaves and extraction to obtain phenolic compounds and saponins for further application in the nutraceutical and pharmaceutical industries.

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Author Contributions

Hong Ngoc Thuy Pham and Van Tang Nguyen collected the material. Quan Van Vuong, Hong Ngoc Thuy Pham, Christopher J. Scarlett, and Van Tang Nguyen designed the experiments. Hong Ngoc Thuy Pham carried out the experiments, analyzed the data and drafted the manuscript. Christopher J. Scarlett, Quan Van Vuong and Michael C. Bowyer contributed to the data interpretation and paper writing. All authors have read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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