

Influence of drying methods on total phenolics, total flavonoids and antioxidant activities in the gurmar leaf (*Gymnema inodorum* (Lour.) Decne.) powder

Amornrat Wongklom*, Natee Banhan, Panukarn Noptalung

Program of Chemistry, Faculty of Science, Ubon Ratchathani Rajabhat University, Ubon Ratchathani, 34000 Thailand

*Corresponding Author: amornrat_dekarnkon@hotmail.com

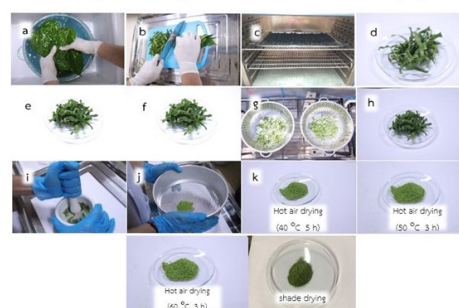
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Abstract

The effect of different drying methods on moisture contents, total phenolics, flavonoids and antioxidant activities in the gurmar leaf (*Gymnema inodorum* (Lour.) Decne.) powder was studied. These drying methods were shade drying and hot air drying at 40 °C – 60 °C for 3 – 5 h. The percentage of moisture content in powder was 3.15 – 4.83 and the water activities were 0.302 – 0.407. Total phenolics, flavonoids, and antioxidant activities of the extract were analyzed. The powder was extracted by distilled water as extracting solvent with controlled temperature 80 °C for 45 min. The results indicated that the hot air-dried powder at 50 °C for 3 h had total phenolics, flavonoids, and antioxidant activities higher than that of the hot air-dried (40 °C for 5 h), hot air-dried (60 °C for 3 h) and shade dried powder, respectively. Total phenolic contents were 20.165, 19.800, 18.115, and 16.903 mg GAE g⁻¹ powder, respectively. Total flavonoid contents were 5.241, 5.238, 5.024, and 4.454 mg CE g⁻¹ powder, respectively. The DPPH free radical scavenging activities were 10.312, 9.684, 9.498, and 8.894 mg AAE g⁻¹ powder, respectively. The ABTS⁺ radical scavenging activity were 8.929, 8.914, 8.844, and 8.819 mg TE g⁻¹ powder, respectively. Total antioxidant capacities were 42.118, 39.639, 38.812, and 37.040 mg AAE g⁻¹ powder, respectively. The reducing powers were 13.195, 13.110, 11.832, and 11.354 mg AAE g⁻¹ powder, respectively. The drying temperature and time affected on the water-soluble phenolics, flavonoids and antioxidant activities in the water extracts of gurmar leaf powder from different drying methods.

Preparation of the gurmar leaf powder by hot air and shade drying



extraction by distilled water



Analysis of active components and antioxidant activities

- Moisture content and water activity
- Total phenolics and flavonoids contents
- DPPH radical scavenging activity
- Total antioxidant capacity
- ABTS radical scavenging activity
- Reducing power

Keywords: Gurmar leaves; Drying methods; Phenolics; Flavonoids; Antioxidant activities

1. Introduction

Gurmar leaves (*Gymnema inodorum* (Lour.) Decne.), called Chiang Da leaf in Thai, can be pounded thoroughly and used as a mask around the head to relieve the symptoms of flu, allergy symptoms, constipation, elimination of toxins in the body, cure asthma, nourish the pancreas to work efficiency and reducing the blood sugar level [1, 2]. Gurmar leaves contain an important substance called gymnemic acid, which has the effect of inhibiting sugar transport, causing the absorption of sugar in the small intestine to slow down. In addition, gymnemic acid also helps to stimulate the secretion of insulin resulting in lower blood sugar levels [3, 4]. There is research showing that this plant is a herb which has properties to reduce blood sugar and has potential for development as a diabetes drug. Active ingredient nourishes the eyes regulating the functions of the body to be normal and it is an antioxidant that causes liver cancer, stomach cancer, coronary artery disease, cataracts in the elderly, and prevent hemolysis, and DNA damage [5]. It can treat diabetes, arthritis, rheumatoid, and gout. Saponin inhibits absorption and lowers sugar levels in the intestines [6, 7].

At present, Gurmar leaves are processed into various products such as tea and beverage products. In particular, it is processed into an herbal tea in leaf and powder form by a simple process. Therefore, Gurmar or Chiang Da leaves tea is produced in many community enterprises through simple drying methods such as sun, and shade drying that are not used drier. However, these drying methods have several limitations due to drying efficiency depending on the weather which the heat level and temperature cannot be controlled, then making it difficult to control the quality of dried herb [8, 9]. In addition, these drying methods are not be able to enough eliminate the percentage of moisture content lower than 10. There is growing of mold and pathogenic microorganisms in leaves or powder tea, resulting in reducing quality of the tea [10 – 14].

Therefore, the researcher interested in studying the drying methods that affect the moisture, phenolics, flavonoids, and antioxidant activities of the gurmar leaf (*Gymnema inodorum* (Lour.) Decne.) powder [15 – 19]. The information on the efficiency and value of the gurmar leaves and can be obtained. These datas can be used as a guideline for the further development such as a commercial gurmar leaves tea product. Mold and pathogenic microorganisms cannot growth on the tea and high active compounds, resulting in high quality leaves or powder tea.

2. Materials and Methods

Preparation of the gurmar leaf powder

The gurmar leaves (*Gymnema inodorum* (Lour.) Decne.) were sampled from Kham Yai Subdistrict, Mueang District, Ubon Ratchathani Province. The leaves were washed with tap water, and allowed to drain. All of them were sliced not to exceed 0.5 cm for width and 400 g of the sliced leaves was weighted, then the sample was dried in the different drying methods as follows; shade drying at $30\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ for 24 h, hot air drying at $40\text{ }^{\circ}\text{C}$ for 5 h, hot air drying at $50\text{ }^{\circ}\text{C}$ for 3 h and hot air drying at $60\text{ }^{\circ}\text{C}$ for 3 h. After drying, the dried leaves were ground and sieved through a 60 mesh sieve. All of them are divided into two parts. The first part was analyzed for moisture contents and water activities analysis, and the second part was stored in a desiccator for determining the active substances and antioxidant activities. Preparation of the gurmar leaf powder was modified following the method reported by Zheng *et al.* [8]. The procedure for preparing the gurmar leaf powder was shown in Fig 1. These datas can be used for the development of a commercial gurmar leaves tea product. These processes consist of washing the herb, slicing the leaves into thin slices, dry, finely grind, pack in suitable packaging and analysis of active compound in product for quality control.

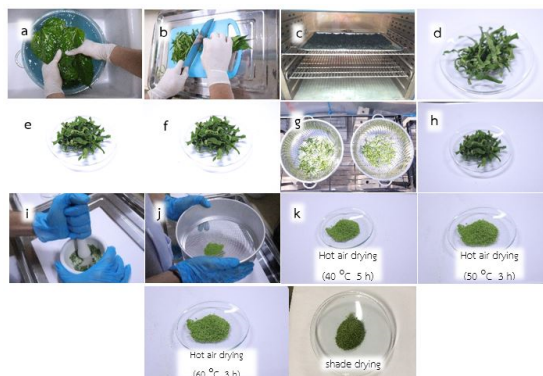


Fig. 1 Preparation of gurmar leaf (*Gymnema inodorum* (Lour.) Decne.) powder; wash the gurmar leaves (a), slice into thin slices (b), hot air drying (c), the hot air dried leaves (d, e, f), shade drying (g), the shade dried leaves (h), grind (i), sieve (j), gurmar leaf (*Gymnema inodorum* (Lour.) Decne.) powder (k).

Preparation of the extract

One gram of the gurmar leaf (*Gymnema inodorum* (Lour.) Decne.) powder was extracted for 45 min at 80 °C using 150 mL of water as solvent [8]. The water extract was filtered through filter paper No. 1. The extract was a yellowish-brown color. The color values of the gurmar leaf powder extract were $L^* 66.46$ $a^* -1.99$ $b^* 23.36$ for hot air dried powder at 40 °C 5 h, $L^* 67.05$ $a^* -3.12$ $b^* 22.72$ for hot air dried powder at 50 °C 3 h, $L^* 66.93$ $a^* -3.64$ $b^* 23.77$ for hot air dried powder at 60 °C 3 h and $L^* 64.08$ $a^* -1.87$ $b^* 26.00$ for shade dried powder.

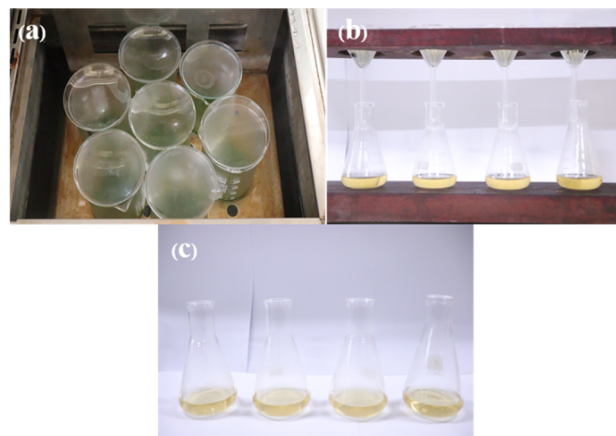


Fig. 2 Preparation of the gurmar leaf powder extract; extraction for 45 min at 80 °C by distilled water (a), filter pass through filter paper (b), The extract was a yellowish brown color (c).

Determination of total phenolics content

Total phenolics contents (TPC) in the gurmar leaf powder extract were determined by the Folin-Ciocalteu colorimetric method [8]. A 1 mL extract was mixed into a series of 15 mL vial with 0.5 mL Folin-Ciocalteu reagent. After sitting for 5 min, the mixture was added with 5 mL 5% ($w v^{-1}$) of Na_2CO_3 , and place for 60 min at room temperature. The absorbance was measured in spectrophotometry at 750 nm. TPC was expressed as milligrams of gallic acid equivalents per gram of powder ($mg\ GAE\ g^{-1}$ powder).

Determination of total flavonoids content

Total flavonoids contents (TFC) of the gurmar leaf powder extract were determined by the formation of an aluminium-flavonoid complex [8]. Briefly, 600 μL water extract was mixed into 2.40 mL distilled water and 180 μL 5% ($w v^{-1}$) of $NaNO_2$ and then shaken as well. After sitting for 5 min, 180 μL 10% ($w v^{-1}$) of $AlCl_3$ was added into the mixture. Next, the mixture was added 1.2 mL of 1 M NaOH and 2.40 mL distilled water. After dark incubation for 5 min, the absorbance was measured at 510 nm and TFC was expressed in milligrams of catechin equivalents per gram of powder ($mg\ CE\ g^{-1}$ powder).

DPPH radical scavenging activity assay

DPPH radical scavenging activities of the gurmar leaf powder extract were determined following the method reported by Zheng *et al.* [8].

Aliquot (0.20 mL) of the extract was mixed to 5 mL of 0.20 g L⁻¹ DPPH solution. After being energetically mixed, the mixture was left in the dark for 30 min. Then, the absorbance was measured at 517 nm. The percentage of inhibition (%I) of DPPH free radical was calculated using the formula:

$$\%I = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

DPPH free radical scavenging activities were expressed as milligrams of ascorbic acid equivalents per gram of powder (mg AAE g⁻¹ powder).

The total antioxidant capacity assay

Total antioxidant capacity assay was based on the reduction of Mo (VI) to Mo (V) with modified by Chahmi *et al.* [9]. A 0.30 mL of the extract was mixed into 3 mL of reagent solution (0.60 mol L⁻¹ sulphuric acid, 28 mmol L⁻¹ of sodium phosphate and 4 mmol L⁻¹ of ammonium molybdate, 1 : 1 : 1). The mixture was incubated at 95 °C for 90 min and then cooled to room temperature. The absorbance was measured at 695 nm [9]. The total antioxidant capacities were expressed as milligrams of ascorbic acid equivalents per gram of powder (mg AAE g⁻¹ powder).

Reducing power assay

Reducing power assay was estimated according to the report of Liu *et al.* [16]. The 1 mL of the extract and ascorbic acid were mixed into 1 mL of sodium phosphate buffer (0.20 M, pH 6.60) and 1 mL of 1% (w v⁻¹) K₃Fe(CN)₆, and incubated in 50 °C for 20 min. After that 1 mL of 10% (w v⁻¹) trichloroacetic acid was added, the mixture was centrifuged at 3700xg for 10 min. Briefly, the 2 mL supernatant was mixed into 2 mL distilled water and 0.4 mL of 0.10% (w v⁻¹) FeCl₃. After 3 – 5 minutes, the absorbance was measured at 700 nm [16]. The reducing powers were expressed as milligrams of ascorbic acid equivalents per gram of powder (mg AAE g⁻¹ powder).

ABTS radical scavenging activity assay

ABTS cation radical activities were assayed by the method of Marecek *et al.* [18]. ABTS

cations radical was generated by reacting 7.40 mM ABTS with 2.40 mM potassium persulfate (1 : 1, v v⁻¹) for 12 – 16 h. Then the mixture was diluted to give an absorbance of 1.00 ± 0.20 at 734 nm. The gurmar leaf powder extract and ascorbic acid standard solution (0.40 mL) were mixed into 4 mL diluted ABTS^{•+} solution and left in dark for 10 min. The absorbance was measured at 734 nm using a spectrophotometer [18]. ABTS radical scavenging activities were calculated and expressed as milligrams of ascorbic acid equivalents per gram of powder (mg AAE g⁻¹ powder).

Moisture content and water activity analysis

Moisture content was analyzed by Leco/TGA701 moisture analyzer and was calculated followed by AOAC [19]. Water activity was analyzed by a water activity meter.

3. Results and Discussion

Moisture content and water activity of the gurmar leaf powder

The gurmar leaves (*Gymnema inodorum* (Lour.) Decne.) were dried by different drying methods as followed shade drying at 30 ± 2 °C for 24 h, hot air drying at 40 °C for 5 h, hot air drying at 50 °C for 3 h, and hot air drying at 60 °C for 3 h, and then ground to fine powder for the analysis of the moisture contents and water activities of the gurmar leaf powder.

The moisture contents and water activities in the gurmar leaf powder from different drying methods were showed in Table 1. The results indicated that drying temperature and time had an effect on moisture contents and water activities [10 – 13]. Fresh gurmar leaves had a percentage of moisture content of 75.87 (a_w 0.962). These leaves were processed by different drying methods, until the moisture contents were reduced and dried. The percentage of moisture content was 3.15 – 4.83. The water activities were 0.302 – 0.407 which is lower than 0.6, which can protect the growth of mold and pathogenic microorganisms in dried leaves or powder [10 – 13].

Table 1 Moisture contents and water activities of the gurmar leaf powder from the different drying methods

drying methods	moisture content (%)	water activities (a_w)
fresh gurmar leaves	75.87	0.962
shade drying (24 h)	4.83	0.407
hot air drying (40 °C 5 h)	3.15	0.302
hot air drying (50 °C 3 h)	3.43	0.386
hot air drying (60 °C 3 h)	3.21	0.371

The gurmar leaf powder from hot air drying methods (40 °C 5 h; 50 °C 3 h; 60 °C 3 h) and shade drying (24 h) had a percentage of moisture content lower than 10 as 3.15, 3.21, 3.43, and 4.83, respectively. The hot air dried gurmar leaf powder (40 °C 5 h; 50 °C 3 h; 60 °C 3 h) had moisture content (3.15 – 3.43%) lower than the shade dried powder (4.83%), due to hot air drying used oven which can control a stable temperature and time at 40 – 60 °C for 3 – 5 h, but shade drying efficiency is depending on the weather which cannot control the heat level and temperature. The drying temperature was average of 30 ± 2 °C and long time for drying, resulting in difficulty to control the quality and moisture contents of dried herb. The research indicated that the temperature and time required for drying affected on moisture contents, when high temperature and long drying times, a moisture content and water activity will be reduced [10 – 13].

Total phenolics and flavonoids content

Total phenolics and flavonoids contents in the gurmar leaf powder by different drying methods were shown in Table 3. The highest of the water-soluble phenolics and flavonoids contents existed in the hot air-dried gurmar leaf powder at 50 °C for 3 h (20.165 ± 0.833 mg GAE g⁻¹ powder; 5.241 ± 0.198 mg CE g⁻¹ powder) among the hot air-dried powder at 40 °C for 5 h (19.800 ± 1.061 mg GAE g⁻¹ powder; 5.238 ± 0.459 mg CE g⁻¹ powder), the hot air-dried powder at 60 °C for 3 h (18.115 ± 0.626 mg GAE g⁻¹ powder; 5.024 ± 0.213 mg CE g⁻¹ powder) and shade dried powder for 24 h (16.903 ± 0.770 mg GAE g⁻¹ powder; 4.454 ± 0.256 mg CE g⁻¹ powder), respectively. Total phenolics and flavonoids contents remained in the hot air-dried powder more than that of the shade dried powder due to the shade drying used for a long time and exposure to UV light, so these active components may be evaporated during the drying process [13]. The research showed that the drying at high temperature and long-time caused the soluble phenolics and flavonoids to evaporate and decomposed during the drying process.

Table 2 Total phenolics and flavonoids contents of the gurmar leaf powder from the different drying methods

drying method	Total phenolics contents (mg GAE g ⁻¹ powder; n = 5)	Total flavonoids contents (mg CE g ⁻¹ powder; n = 5)
shade drying (24 h)	16.903 ± 0.770	4.454 ± 0.256
hot air drying (40 °C 5 h)	19.800 ± 1.061	5.238 ± 0.459
hot air drying (50 °C 3 h)	20.165 ± 0.833	5.241 ± 0.198
hot air drying (60 °C 3 h)	18.115 ± 0.626	5.024 ± 0.213

The DPPH free radical scavenging activity, ABTS free radical scavenging activity, total antioxidant capacity, and reducing power

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution. This free radical is stable at room temperature and can reduce in the presence of an antioxidant molecule, giving rise to colorless solution. DPPH radical is a stable free radical that can donate hydrogen when reacts with antioxidant compounds and reduce to diphenyl picrylhydrazine. These showed the ability of extracts to neutralize free radicals which possess unpaired electrons. The DPPH free radical scavenging activities in the gurmar leaf powder by different drying methods were shown in Table 3. The hot air-dried powder (40 – 60 °C for 3 – 5 h) had DPPH free radical scavenging activities more than the shade-dried powder. The highest of DPPH free radical scavenging activities existed in the hot air-dried powder at 50 °C for 3 h (10.312 ± 0.487 mg AAE g⁻¹ powder) among the hot air-dried at 40 °C for 5 h (9.684 ± 0.885 mg AAE g⁻¹ powder), 60 °C 3 h (9.498 ± 0.544 mg AAE g⁻¹ powder) and shade dried for 24 h (8.894 ± 0.458 mg AAE g⁻¹ powder), respectively. The gurmar leaf powder that has been dried by shade drying (30 ± 2 °C) for 24 h as long-term drying, total phenolics and flavonoids evaporated with water and exposed a UV light during the drying process, causing the amount of these components decreased. As a result, the DPPH antioxidant activity was reduced as well. An increase of the DPPH inhibition caused by antioxidant might be the scavenging ability of radicals by hydrogen donation. It can also be seen that the water extract was active with relation to the water-soluble phenolics, flavonoids and phytochemicals in the gurmar leaf powder. Therefore, it can be deduced that drying methods, drying temperature and time had an effect on the antioxidant activities of the gurmar leaf powder. The DPPH antioxidant activity was related to the phenolics and flavonoids contents, because these active components are capable of contributing a hydrogen radical to free radicals.

In addition, drying has an effect on the DPPH antioxidant activity. Long-term drying showed the results in lower DPPH antioxidant activity [13 – 18].

The ABTS⁺ free radical activities measure the relative ability of antioxidants to scavenge the ABTS generated in aqueous phase, as compared with a Trolox standard. The ABTS is generated by reacting with a strong oxidizing agent (potassium persulfate) with the ABTS salt. The reduction of the blue-green ABTS radical by hydrogen-donating antioxidants is measured by the suppression of its characteristic long wave absorption spectrum. The ABTS⁺ radical scavenging activities in the gurmar leaf powder were shown in Table 3. The highest of ABTS⁺ radical scavenging activity existed in the hot air-dried powder at 50 °C for 3 h (8.929 ± 0.337 mg TE g⁻¹ powder) among the hot air dried powder at 40 °C for 5 h (8.914 ± 0.312 mg TE g⁻¹ powder), the hot air dried powder at 60 °C for 3 h (8.844 ± 0.179 mg TE g⁻¹ powder) and the shade dried powder (8.819 ± 0.245 mg TE g⁻¹ powder), respectively. These results could be hypothesized that the hot air-dried gurmar leaf powder had higher water-soluble phenolics, flavonoids, and phytochemicals contents than the shade dried powder (24 h). The results found that the ABTS⁺ radical scavenging activity related to the water-soluble phenolics, flavonoids and phytochemicals contents, because of active compounds can give hydrogen radical to ABTS free radicals that ABTS is not free radicals. In addition, drying temperature and time also have an effect on ABTS antioxidant activities [13 – 18].

The phosphomolybdenum was a quantitative assay. Since the antioxidant activity was expressed as number of ascorbic acid, the antioxidant capacity assay was based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate Mo (V) complex at acid pH. The total antioxidant capacities in the gurmar leaf powder were shown in Table 3. The highest of total antioxidant capacities existed in the hot air-dried powder at 50 °C for 3 h (42.118 ± 2.043 mg AAE g⁻¹ powder) among the hot air-dried powder at 40 °C for 5 h (39.639 ± 1.387 mg

AAE g⁻¹ powder), 60 °C for 3 h (38.812 ± 0.827 mg AAE g⁻¹ powder) and the shade dried powder (37.040 ± 2.132 mg AAE g⁻¹ powder), respectively. An increase in the total antioxidant capacities relates to the water-soluble phenolics, flavonoids, and phytochemicals contents in the gurmar leaf powder [14 – 18].

Reducing power was assessed by ferric to ferrous ion reduction assay. Reducing power assay method is based on the principle that the reduction potential substances, reacting with potassium ferricyanide (Fe³⁺) to form potassium ferrocyanide (Fe²⁺), which then reacts with ferric chloride to form ferric–ferrous complex that has an absorption maximum at 700 nm. Reducing power of the gurmar leaf powder was shown in Table 3. The highest of reducing power existed in the hot air-dried gurmar leaf powder at 50 °C for 3 h (13.195 ± 0.439 mg AAE g⁻¹ powder) among the hot air-dried powder at 40 °C for 5 h (13.110 ± 0.275 mg AAE g⁻¹ powder), the hot air-dried powder at 60 °C for 3 h (11.832 ± 0.674 mg AAE g⁻¹ powder) and the shade dried powder (11.354 ± 1.066 mg AAE g⁻¹ powder), respectively. The hot air-dried powder (50 °C for 3 h) had the higher value of water-soluble phenolics, flavonoids, and phytochemicals contents than the hot air-dried powder at 40 °C for 5 h, 60 °C for 3 h and shade dried powder.

The reducing power is related to total phenolics, flavonoids and phytochemicals contents because these antioxidants that can give free electrons in the reduction reaction, the antioxidants give electrons to ferric ion [14 – 18].

The study found that drying method, temperature and drying time influenced moisture content, active substances and antioxidant activities in gurmar leaf powder. The sun and shade drying method cannot control the drying temperature and time. The drying condition is depending on the weather, then it takes a long drying time corresponding to A. Wongklom reported the using the sun drying method led to takes for 49 h for drying time and effect on the water soluble total phenolics and flavonoids contents, and antioxidant activities of sunchoke (*Helianthus tuberosus* L.) powder [13]. In addition, the hot air drying method can control the drying temperature and time, because it uses a hot air dryer. Using high drying temperature and a long time reduced active substances and antioxidant activities in gurmar leaf powder corresponding to M. Saifullah *et al.* reported that using a high temperature and long time for drying led to reduced extractable phenolic compounds and antioxidant properties from lemon myrtle dried leaves [14].

Table 3 DPPH free radical scavenging activities, ABTS free radical scavenging activities, total antioxidant capacities and reducing powers of the gurmar leaf powder from the different drying methods

drying method	DPPH free radical scavenging activity (mg AAE g ⁻¹ powder; n = 7)	ABTS free radical scavenging activity (mg TE g ⁻¹ powder; n = 7)	total antioxidant capacity (mg AAE g ⁻¹ powder; n = 7)	reducing power (mg AAE g ⁻¹ powder; n = 7)
shade drying (24 h)	8.894 ± 0.458	8.819 ± 0.245	37.040 ± 2.132	11.354 ± 1.066
hot air drying (40 °C 5 h)	9.684 ± 0.885	8.914 ± 0.312	39.639 ± 1.387	13.110 ± 0.275
hot air drying (50 °C 3 h)	10.312 ± 0.487	8.929 ± 0.337	42.118 ± 2.043	13.195 ± 0.439
hot air drying (60 °C 3 h)	9.498 ± 0.544	8.844 ± 0.179	38.812 ± 0.827	11.832 ± 0.674

4. Conclusion

The drying methods include shade drying, hot air drying at 40 °C for 5 h, hot air drying at 50 °C for 3 h, and hot air drying at 60 °C for 3 h, were affected on moisture contents, the water-soluble total phenolics, flavonoids, and phytochemical contents, and antioxidant activities of the gurmar leaf powder. The moisture contents were lower than 10% and water activities were lower than 0.6, which can protect the growth of mold and pathogenic microorganisms in the powder. The hot air-dried (50 °C for 3 h) gurmar leaf powder had total phenolics, flavonoids, and antioxidant activities higher than the hot air-dried (40 °C for 5 h and 60 °C for 3 h) and shade dried powder, respectively. The drying temperature and time affected on the water-soluble total phenolics, flavonoids, and antioxidant activities in the gurmar leaf powder from different drying methods. These results indicated that the hot air-drying at 50 °C for 3 h was a proper method for drying the gurmar leaf to tea product. There is not growing of microorganisms and high active compounds, resulting in high quality tea.

In addition, the gurmar leaf powder also be used to study the gymnemic acid content, which has the effect of inhibiting sugar transport and effects of lowering blood sugar levels, and clinical testing in the further.

5. Acknowledgement

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6. References

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