SNRU Journal of Science and Technology

Journal home page: snrujst.snru.ac.th

Effect of drying methods on antioxidant capacity, total phenolic and flavonoid contents of Phakwan (*Sauropus androgynus* (L.) Merr.) powder

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Received: 10 July 2017; **Revised:** 29 October 2017; **Accepted:** 25 January 2018; **Available online:** 1 May 2018

Abstract

Effect of three different drying methods on antioxidant capacity, total phenolic and flavonoid contents of Phakwan (*Sauropus androgynus* (L.) Merr.) powder were investigated. Samples were dried with three difference methods (sun drying, oven drying and sun-oven-drying), then ground and extracted with methanol as crude extract. DPPH free radical scavenging activity, total antioxidant capacity and reducing power were used as markers for antioxidant capacity. TPC were measured using the Folin-Ciocalteau assay. TFC were assessed using based on the formation of a complex flavonoidaluminium. The oven-dried samples had TPC (23.37 ± 0.70 mg GAE g^{-1} dry extract), total antioxidant capacity (120.10 ± 9.23 mg AE g^{-1} dry extract and 229.05 ± 8.55 mg BHTE g^{-1} dry extract) and reducing power higher than that of the sun-dried and sun-oven-dried samples while the sun-oven-dried samples had TFC and DPPH free radical scavenging activity higher than that of oven-dried and sun-dried samples. There was no significant difference in efficiency between sun and sun-oven-drying methods. The variation of antioxidant capacity, TPC and TFC of Phakwan (*Sauropus androgynus* (L.) Merr.) powder are due to the effect of the three difference drying methods.

Keywords: drying method; antioxidant capacity; total phenolics; total flavonoids

1. Introduction

The food industry is increasingly interested in herbs, due to growing of consumer demands for healthy foods from natural sources [1]. Star gooseberry is normal name of *Sauropus androgynus* (L.) Merr locally called "Phakwan". They are widely distributed in the all part of Thailand and mostly found in the combination of soaked clay, wet evergreen forest or grove wood. It is an herbaceous perennial plant which belongs to the family of Euphorbiaceae. They are not only used for food, but also for other purposes, including medicine, anti-inflammatory and antioxidant activities herb. It is well known that phenolics and flavonoids are important antioxidant substances obtained from most natural plants. These substances are able to reduce free radicals like superoxide, peroxyl, alkoxylate and hydroxyl [2, 3]. Plant consists of a set of organs whose growth depends on the environmental conditions in which it develops, including the intercepted light energy, water and available nutrients drawn from the soil [3]. The plants are mostly made for the antioxidant beverages, herbal tea or infusion herbal tea [1, 4].

Immediately after harvesting, these highly perishable raw materials have to be preserved against deterioration and spoilage. Fresh herbs usually contain 75 – 80% moisture, and the moisture levels need to be lowered to less than 15% for their preservation. Drying is by far the most widely used treatment of herbs for inhibiting microbial growth and forestalling certain biochemical changes but, at the same time, it can give rise to other alternations that affect herb quality, such as changes in appearance and alterations caused by losing in antioxidant or the formation of new substance as a result of oxidation reactions or esterification reactions. Most

studies have reported changes in color and active compound of the herbs after drying [1, 4]. Thus, this study was planned to evaluate the antioxidant properties of the *Sauropus androgynus* (L.) Merr. powder from three different drying methods; sun-drying, oven-drying, sun and oven-drying on total phenolics, total flavonoids contents, antioxidant capacity, and total antioxidant capacity. The antioxidant capacity and total antioxidant capacity were measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, phosphomolybdenum and reducing power assays, respectively. The organic solvent showed a significant influence on the concentration of antioxidants. In this study, we investigated the methanol extracts [2, 5, 6]. The results of this study will allow us to optimize the possibility of exploiting this plant to produce antioxidants agents used in the global effort to combat free radical damages. In the further, Phakwan (*Sauropus androgynus* (L.) Merr.) was processed to herbal tea or infusion herbal tea as community products.

2. Materials and methods

Reagents and standards

DPPH was purchased from Sigma-Aldrich (St. Louis, MO, USA). Butylated hydroxytoluene (BHT), ascorbic acid, ammonium molybdate, sodium phosphate, sulphuric acid, gallic acid, $FeCl_3$, $K_3Fe(CN)_6$, Sodium carbonate and Folin-Ciocalteu reagent (FCR) were purchased from Merk (Darmstadt, Germany). All the other chemicals and solvents used were of analytical grade.

Plants materials and preparation of extracts

Phakwan (*Sauropus androgynus* (L.) Merr.) was collected in April to May 2015 from Ban Khamplalai, Sawangvirawong, Ubonratchathani province of Thailand (Fig. 1). Samples were dried with three difference methods as I) sun-drying method (various temperature; 28 hrs.), II) oven-drying method (60 °C; 16 hrs.) and III) sun (various temperature; 14 hrs.) and oven (60 °C; 8 hrs.) drying method until the moisture was reduced to less than 15% and then ground. Methanol extraction was performed by maceration at the ratio of 15% (w v^{-1}) for 3 days for three times under room temperature for 300 g plant powder, then the extract was concentrated in vacuum at 50 °C to obtain a dry methanol extract, then stored at 4 °C for further use.

Fig. 1 Phakwan (*Sauropus androgynus* (L.) Merr.)

DPPH scavenging activity

The hydrogen atoms or electrons donation ability of the plant extracts and some pure compounds were measured from the bleaching of a purple-coloured methanol solution of DPPH [7 – 9]. Briefly, 3.90 mL of a 0.025 mg L^{-1} solution of DPPH radical in methanol was added to 0.10 mL of the extract at different concentrations. The solutions were shaked and incubated for 30 min in dark. The absorbance of the resulting solution was measured at 515 nm with a spectrophotometer (Perkin elmer 3100, USA) [2, 5]. The percentage inhibition of activity was calculated as:

$$\%\text{Inhibition} = (A_{\text{blank}} - A_{\text{sample}}) \times 100 / A_{\text{blank}} \quad (1)$$

Ascorbic acid was used as positive control and the concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against the extract concentration.

Iron (III) to Iron (II) capacity

The reductive capacity of the extract was determined using ferric to ferrous iron reduction assay as determined spectrophotometrically from the formation of Perl's Prussian blue coloured complex [10]. Briefly, 1 mL of each methanolic extract solutions was mixed with 2.50 mL of phosphate buffer (0.20 mol L^{-1}, pH 7.0) and 2.5 mL of potassium hexacyanoferrate solution (1% w v^{-1}). After 30 min incubation at 50 °C, 2.50 mL of trichloroacetic acid (10% w v^{-1}) was added to the mixture. Then, 2.50 mL of this solution was mixed with 2.50 mL of distilled water and 0.50 mL of $FeCl_3$ solution (0.1% w v^{-1}), and the absorbance was measured at 700 nm. BHT and ascorbic acid standards were used for comparison [7].

Determination of total antioxidant capacity

The assay was based on the reduction of Mo (VI) to Mo (V) and subsequent formation of a green phosphate/Mo(V) complex in acid pH [8, 9]. A 0.3 mL of methanol extract solution was added to 3 mL of reagent solution (0.60 mol L^{-1} sulphuric acid, 28 mmol L^{-1} sodium phosphate and 4 mmol L^{-1} ammonium molybdate, 1:1:1). The mixtures were incubated at 95 °C for 90 min and then cooled to room temperature. The absorbance was measured at 695 nm. The total antioxidant activity was expressed as the number of equivalence of ascorbic acid and BHT standard [7].

Determination of total phenolic compounds content

The total phenolic content was determined using the FCR [10, 11]. The reaction mixture contained 100 µL of methanolic solution (1 mg mL^{-1}) of the extract, 0.50 mL of FCR, 1.50 mL of 10% (w v^{-1}) sodium carbonate and 10 mL of distilled water. After 2h of reaction at ambient temperature, the absorbance was measured at 765 nm and used to calculate the phenolic contents, using gallic acid as a standard [7]. Then the total phenolic contents were expressed in term of gallic acid equivalents (mg GAE/g dry extract).

Total flavonoids contents

The flavonoid contents of the methanolic extracts were assessed using the method of Lamaison and Carnat based on the formation of a complex flavonoidaluminium [12]. Briefly, 1 mL of diluted methanolic extract (50 mg L^{-1}) was mixed with 1 mL of 2% (w v^{-1}) aluminium chloride methanolic solution, after incubation for 10 min at room temperature. The absorbance was measured at 430 nm and the flavonoids content was expressed in mg quercetin equivalent (QE) per gram of dry extract.

Statistical analysis

The measurements of total phenolic compounds, total flavonoids and DPPH radical-scavenging activity, total antioxidant and reducing capacity were carried out between three and seven replicates. The results are expressed as mean ±SD.

3. Results and discussion

Extraction yield

As shown in Table 1, the extractive solvent was methanol. The highest extraction yield of *Sauropus androgynus* (L.) Merr. extract was the oven-dried powder (9.42% moisture) with 33.17% (percentage of dry extract, w w^{-1}), followed by the sun-dried powder (9.55% moisture) with 29.35% and the sun and oven-dried powder (10.036% moisture) with 29.34%.

Table 1 Residues yields (% of dry extract) of methanol extract of *Sauropus androgynus* (L.) Merr. powder from three difference drying methods

drying method	Yields (%)
Sun dried	29.35
Oven dried	33.17
Sun and oven dried	29.34

DPPH scavenging activity

In this study, *Sauropus androgynus* (L.) Merr. powder from three difference drying methods were investigated for their antioxidant activity with DPPH scavenging assay. Results showed an important antioxidant power of *Sauropus androgynus* (L.) Merr. powder compared to the ascorbic acid standard (Fig. 2). The antioxidant activity of all powder samples increased with the increasing of their concentrations. The sun and oven-dried powder was more effective than the sun-dried and oven-dried powder, respectively. The IC_{50} value was defined as the concentration of sample that scavenged 50% of the DPPH. The antioxidant activity of the sun and oven-dried powder (IC_{50} value of 15.77 mg mL^{-1}) was superior to all samples but lower than the positive control which was not near to the inhibition capacity of the positives controls BHT (IC_{50} = 0.15 mg mL^{-1}) and ascorbic acid (IC_{50} = 0.12 mg mL^{-1}) [7], followed by the oven-dried powder (IC_{50} = 25.19 mg mL^{-1}) then the sun-dried powder(IC_{50} = 25.46 mg mL^{-1}) (Table 2).

Table 2 IC_{50} (mg mL^{-1}) values of *Sauropus androgynus* (L.) Merr. powder from the three difference drying methods to DPPH assay.

drying method	IC_{50} (mg mL^{-1})
Sun dried	25.46
Oven dried	25.19
Sun and oven dried	15.77

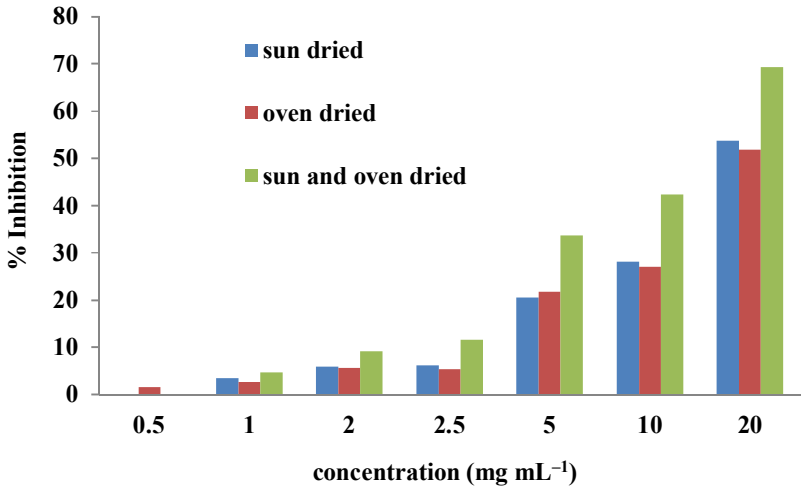

Fig. 2 Scavenging activities of methanol extracts of different concentrations of *Sauropus androgynous* (L.) Merr. powder from three difference drying methods on the DPPH assay.

Determination of total antioxidant capacity

The phosphomolybdenum was a quantitative assay. Since the antioxidant activity was expressed as number of ascorbic acid and BHT, the method 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 [7]. The three difference drying methods had different levels of antioxidant capacity (Table 3). The oven-dried powder had a higher capacity than the sun-dried powder and the sun and oven-dried powder. The oven-dried *Sauropus androgynus* (L.) Merr. powder had the most important value with 120.10 ± 9.23 mg ascorbic acid equivalent/g dry extract and 229.05 ± 8.55 mg BHT equivalent/g dry extract, followed by the sun and oven-dried powder and the sun dried powder.

Table 3 Total antioxidant capacities of *Sauropus androgynus* (L.) Merr. powder from the three difference drying methods.

drying method	Equivalent to ascorbic acid (mg g^{-1} dry extract, n=5)	Equivalent to BHT (mg g^{-1} dry extract, n=5)
sun dried	86.14 ± 3.69	155.03 ± 3.18
oven dried	120.10 ± 9.23	229.05 ± 8.55
sun and oven dried	103.31 ± 4.32	205.19 ± 5.79

Reductive capacity

Generally, the reducing properties associated with the presence of compounds which exerted their action by breaking the free radical chain via donating a hydrogen atom [13]. In the ferric to ferrous iron reduction assay, the electron donation capacity of the extracts was assessed and compared to that of ascorbic acid and BHT. The strong reducing agent (Fig. 3) showed the iron (III) to iron (II) reductive activities for the *Sauropus androgynus* (L.) Merr. powder from three difference drying methods. The reducing power of all powder samples increased with the increasing of their concentrations. The oven-dried *Sauropus androgynus* (L.) Merr. powder was the most active one among the three difference methods, the sun-dried powder sample showed the lowest reductive power in the assay.

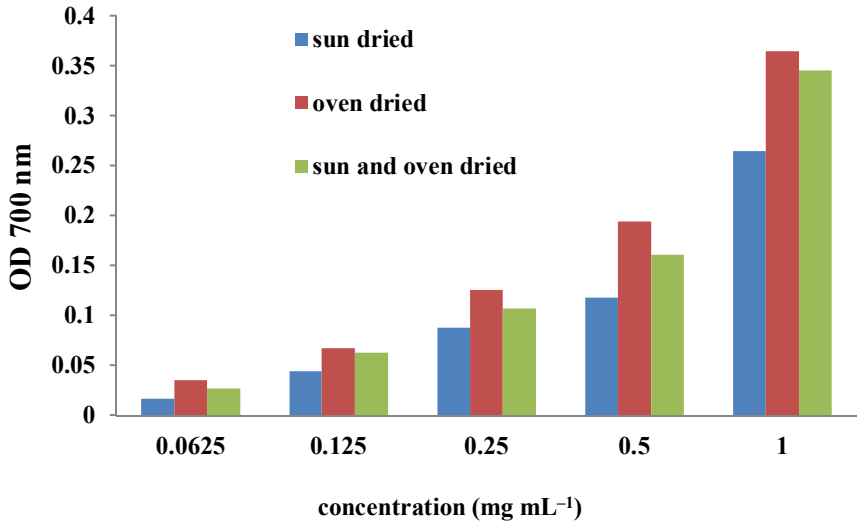

Fig. 3 The iron (III) to iron (II) reductive activities for the *Sauropus androgynus* (L.) Merr. powder from three difference drying methods compared to that of ascorbic acid and BHT standard. Assay performed in three replicates.

Total phenolics and flavonoids contents

The *Sauropus androgynus* (L.) Merr. powder had an important charge of phenols and flavonoids. Their values varied widely for powder samples from the three difference drying methods. Total phenolic contents were ranging from 14.20 to 23.37 mg GAE g^{-1} dry extract. The highest amount of total phenolic compounds was found in oven-dried powder with 23.37 ± 0.70 mg GAE g^{-1} dry extract, followed by the sun and oven-dried powder with 19.40 ± 1.09 mg GAE g^{-1} dry extract and the sun-dried powder with 14.20 ± 0.54 mg GAE g^{-1} dry extract. In addition, total flavonoid contents have been found ranging from 9.52 to 15.63 mg QE g^{-1} dry extract for the *Sauropus androgynus* (L.) Merr. powder from the three differences drying methods (Table 4). The highest amount of total flavonoid contents was found in the sun and oven-dried powder with 15.63 ± 0.94 mg QE g^{-1} dry extract, followed by the oven-dried powder with 12.54 ± 0.90 mg QE g^{-1} dry extract and the sun-dried powder with 9.52 ± 0.74 mg QE g^{-1} dry extract.

Table 4 Total phenolic and total flavonoid contents of the *Sauropus androgynus* (L.) Merr. powder from three difference drying methods

drying method	Total phenolic contents (mg GAE g^{-1} dry extract; n = 7)	Total flavonoids contents (mg QE g^{-1} dry extract; n = 7)
sun dried	14.20 ± 0.54	9.52 ± 0.74
oven dried	23.37 ± 0.70	12.54 ± 0.90
sun and oven dried	19.40 ± 1.09	15.63 ± 0.94

4. Conclusion

In order to show the antioxidant potentials of *Sauropus androgynus* (L.) Merr. and to ascertain the conditions of the difference drying methods in its chemical constituents, the present work was the comprehensive study of phenolics, flavonoids, and antioxidant activity of the *Sauropus androgynus* (L.) Merr. from Ubonratchathani of Thailand. It is extremely important to point out that, there was a correlation between antioxidant activity potential and amount of phenolic and flavonoid compounds in all powder samples from the difference drying methods, in agreement with the previous investigation. The phenolic content estimated in our results was probably responsible for the total antioxidant capacity and reducing power of the *Sauropus androgynus* (L.) Merr. powder. In this study, total phenolic compounds were attributed to the overall antioxidant activities and they have reserved attention because of their physiological function, including cardio protective action and hepatoprotective activity mainly attributed to the antioxidant potential that might occur by reduction of lipid peroxidation and cellular damage. This study showed that the flavonoid content did vary relative to the difference drying methods of *Sauropus androgynus* (L.) Merr. powder. Flavonoids and phenolic compounds were important components which could be used for the free radical-scavenging activity. Comparing the plant from the three difference drying methods, we found that total phenolic contents exhibited the descending order: oven-dried powder followed by the sun and oven-dried powder then the sun-dried powder. The oven drying method could protect the decomposition of TP and binding by other compound while TP were decomposed by photochemical reaction in the sun drying and sun-oven drying method. These results showed that the total phenolic contents have an obvious variation according to the drying method. The oven dried *Sauropus androgynus* (L.) Merr. powder had the greatest antioxidant properties in total antioxidant capacity and reductive power of assays, and the highest total phenolic contents that have been considered powerful antioxidants *in vitro* and proved to be more potent antioxidants. The plant *Sauropus androgynus* (L.) Merr. is a good source of phenols and natural antioxidants that might have benefits for health and suitable processed to herbal tea or infusion herbal tea.

The total phenolics, total flavonoids, antioxidant capacity and reducing power assays showed a high degree of correlation. The highest correlation was observed between the total phenolics, total

antioxidant capacity and reducing power values. This was expected that all assays had the same principle of electron-transfer (ET)-based antioxidation reactions. A number of authors [2, 5, 7, 8] reported high correlation between total phenolics and antioxidant capacity assays. These results suggest that the TP and TF are the key contributors to the antioxidant capcity of the *Sauropus androgynus* (L.) Merr. extract.

Drying of herbs has been found to be useful technique for increasing the amount of phenolic compounds and antioxidant capacity of the herb samples [14, 15]. The oven-drying was found to be the best method for all the samples. The sun-drying and sun-oven-drying showed a similar efficiency in extraction antioxidant compounds from *Sauropus androgynus* (L.) Merr. powder.

5. Acknowledgement

This work was supported by Faculty of Science, Ubon Ratchathani Rajabhat University of Thailand.

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