



Effect of the drying methods on total phenolics, total flavonoids and antioxidant activities in Ya-Nang leaf (*Tiliacora triandra*) powder

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Abstract

The objective of this research was to study the effect of different drying methods on the amount of total phenolics, flavonoids and antioxidant activity in the Ya-Nang leaf powder using 2 drying methods, were 1) sun drying 2) hot air drying (50 °C 6 h, 60 °C 5 h, 70 °C 4 h) used distilled water as a solvent, then extracted under temperature 80 °C for 45

min. The extract was analyzed for total phenolics content, total flavonoids content and antioxidant capacity measured using DPPH free radical scavenging activity, ABTS free radical scavenging activity, total antioxidant capacity and reducing power. The results found that the drying process at hot air 60 °C for 5 h shown the best condition of total phenolics content (11.277 ± 0.158 mg GAE g⁻¹ powder), total flavonoids content (9.485 ± 0.362 mg CE g⁻¹ powder), DPPH free radical scavenging activity (23.293 ± 0.352 mg AAE g⁻¹ powder), ABTS free radical scavenging activity (10.155 ± 0.393 mg AAE g⁻¹ powder), total antioxidant capacity (32.382 ± 0.414 mg AAE g⁻¹ powder) and reducing power (16.037 ± 0.039 mg AAE g⁻¹ powder). The results indicated the appropriate drying method at 60 °C for 5 h made the Ya-Nang leaf powder contains high bioactive compounds and antioxidant activities. It can use for further development of commercial Ya-Nang tea products.

Keywords: drying method; antioxidant; Ya-Nang leaf

Preparation of the Ya-Nang leaf (*Tiliacora triandra*) powder by hot air and sun drying



Analysis of active components and antioxidant activities

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

1. Introduction

Bamboo grass or Ya-Nang in scientific name is *Tiliacora triandra*. (Colebr.) diels, family name is Menispermaceae, as well as wormwood, koklan, and blood soap. Ya-Nang looks like a vine related to other wood. It is a popular Thai herb. “Ya Nang” is another useful vegetable and high nutritional value. Ya-Nang is a kind of medicinal plant with

various medicinal properties such as anti-fever, anti-alcohol poisoning, anti-malaria, antioxidant, anti-inflammation, lowering blood sugar levels and neuroprotective [1, 2]. The active ingredient from Ya-Nang leaf extract has been studied and found that it contains phenolic compounds and high amounts of flavonoids such as gallic acid and quercetin contents. Quercetin can reduce the lower blood glucose in induced diabetes mellitus by inhibiting alpha-glucosidase

activity. It inhibits the digestion of carbohydrates, reduces glucose absorption in the small intestine [3, 4], increase the response of cells to insulin and increase glucose tolerance [4]. Beside, gallic acid has been reported to induce insulin secretion and protect the pancreatic beta cell damage in diabetic induced rats. It used in cooking many local recipes and contains the antioxidants such as beta-carotene, xanthophyll, vitamin C, vitamin E, tannins and phenolic compounds [3 – 5]. There are various properties because it is a cold herb, contains natural chlorophyll. It is a popular herb used as a seasoning helps increase the mellowness of food such as bamboo shoot curry, bamboo shoot soup, sweet curry, herb juices, mixed herb tea products and others. [6 – 8]. Currently, Ya-Nang leaves are processed into various products including Ya-Nang leaf tea and beverage products. In particular, it is used to produce Ya-Nang leaf tea, both in tea leaf and powder form. Because, the processing of Ya-Nang leaf tea is a simple process. Therefore, Ya-Nang leaf tea is produced and sold in many community enterprises which each area has a different drying method. The drying methods are sun, shade and hot air or oven drying. It takes different drying time and temperature. Such drying method and condition may not be able to completely remove the water content from Ya-Nang leaf or herbs may effect on the quality of herb. If the moisture content is greater than 10%, microorganisms or fungi may develop in the herbal leaves or powder [9]. The active substances contents in tea may deteriorate. The quality of the tea produced has decreased and cannot be sold.

Therefore, this study aimed to investigate the effect of the different drying methods on total phenolics, total flavonoids and

antioxidant activities in the Ya-Nang leaf (*Tiliacora triandra*) powder [10]. The drying methods were sun and hot air drying. The different time and temperature for drying were used. These studies were to obtain information on the optimum drying conditions and quality of the Ya-Nang leaf powder used for processing into products. It will be able to use as a guideline for further development of commercial Ya-Nang tea products.

2. Materials and Methods

Sample collection and preparation of Ya-Nang leaf powder

Ya-Nang leaves (*Tiliacora triandra*) were collected from Ban Nikhom, Rai Noi Subdistrict, Mueang District, Ubon Ratchathani Province, Thailand. The leaves were cleaned by tap water three times, then allowed to drain and sliced to a width that does not exceed 0.5 cm. All of them were weighed to 200 g to be dried in different drying methods as follows; 1) sun drying (18 h; 29 – 34 °C) 2) hot air drying at 50 °C for 6 h, 3) hot air drying at 60 °C for 5 h and hot air drying at 70 °C for 4 h, by hot air circulating in the cabinet at a wind speed of 1.5 m s⁻¹ per square meter of the surface area of the tray and have a pipe system to direct the hot air upward through each tray to distribute the hot air evenly.

After drying, the dried leaves were ground into the powder and sieved through a 60-mesh sieve. All of them were kept under 4 °C before analysis. Total phenolics, total flavonoids, DPPH free radical scavenging activity, ABTS free radical scavenging activity, total antioxidant capacity, and reducing power were analyzed in the Ya-Nang leaf (*Tiliacora triandra*) powder. The procedure for preparing the leaf powder was shown in **Fig. 1**.



Fig. 1 Preparation of the Ya-Nang leaf powder; wash the Ya-Nang leaves (a), slice into thin slices (b), hot air drying (c), the hot air dried leaves (d, e, f), sun drying (g), the sun dried leaves (h), grind (i), sieve (j), the Ya-Nang leaf powder (k, l, m, n)

Preparation of extract

In accordance with the objective to produce the Ya-Nang leaf tea, the water-soluble active components were extracted with modified method from Zheng *et al.* [11]. One gram of the dried Ya-Nang leaf powder was extracted for 45 min at 80 °C using 150 mL of distilled water. The water extract was filtered pass through filter paper No.1 and then the extract

observed to clear the solution. The Ya-Nang leaf powder extract was a brownish yellow color and used for analyze the contents of water-soluble active components, namely total phenolics, flavonoids, as well as antioxidant activites. The procedure for preparation of the Ya-Nang leaf powder extract was shown in **Fig. 2**.

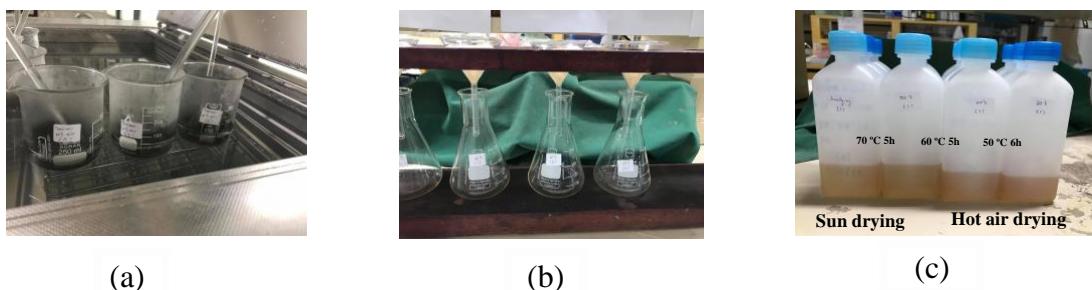


Fig. 2 Preparation of the Ya-Nang leaf powder extract; extraction for 45 min at 80 °C by distilled water (a), filter pass through filter paper (b), The extract was a yellowish brown color (c)

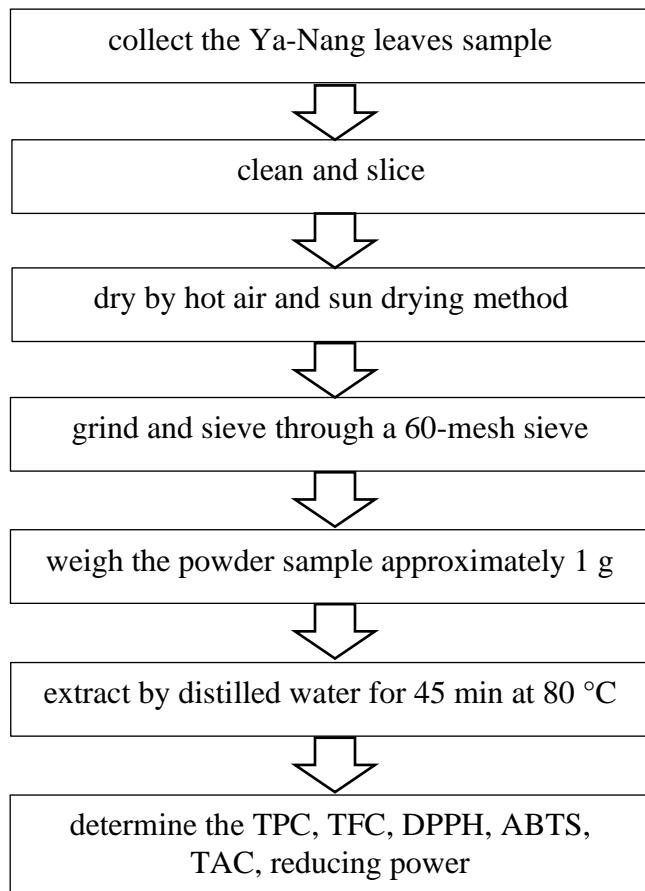


Fig. 3 The flowchart of analysis of active components and antioxidant activities in the Ya-Nang leaf powder

Determination of total phenolics content

Total phenolics content (TPC) in the Ya-Nang leaf powder was determined by the Folin-Ciocalteu colorimetric method with modified method from Bei *et al.* [12]. A 1 mL extract and 1 mL of gallic acid standard solution were mixed into a series of the 15 mL vial with 0.5 mL Folin-Ciocalteu reagent, shook for 1 min with a vortex mixer. After leaving at room temperature for 5 min, the mixture was added with 1.5 mL 20% (w v⁻¹) Na₂CO₃, then added with 7 mL distilled water. All of them were shaken and placed for 60 min at room temperature. The absorbance of the extract and standard solution was measured at 760 nm in spectrophotometer. TPC was calculated by the absorbance of extract according to the standard curve as milligrams of gallic acid equivalents per gram of powder (mg GAE g⁻¹ powder).

Determination of total flavonoids content

Total flavonoids content (TFC) of the Ya-Nang leaf powder extract was determined by an aluminium chloride colorimetric method with modified method from Deseo *et al.* [13]. The extract was determined by the formation of an aluminum-flavonoid complex. Briefly, 1 mL of water extract and catechin standard solution were mixed into 4 mL distilled water, 0.3 mL 5% (w v⁻¹) NaNO₂ and shook well. After leaving for 5 min, 0.3 mL of 10% (w v⁻¹) AlCl₃ was added into the mixture, following 2 mL 1 M NaOH and 2.40 mL distilled water. After leaving for 5 min, the absorbance was measured at 510 nm and TFC was expressed in milligrams of catechin equivalents per gram of powder (mg CEg⁻¹ powder).

DPPH scavenging activity assay

DPPH radical scavenging activity of the Ya-Nang leaf powder water extract was determined following the method which reported by Zheng *et al.* [11]. The method base on the scavenging of DPPH by antioxidants, which upon a reduction reaction decolorizes the DPPH methanol solution. The assay measures the reducing ability of antioxidants toward the DPPH radical. An aliquot (0.20 mL) of the water extract and ascorbic acid standard solution was mixed to 5 mL of 0.20 g L⁻¹ DPPH solution. After being energetically mixed, the mixture was lifted in the dark at room temperature 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_{blank} – A_{sample}) / A_{blank}] × 100. DPPH free radical scavenging activity was expressed as milligrams of ascorbic acid equivalents per gram of powder (mg AAE g⁻¹ powder).

ABTS scavenging activity assay

ABTS cation radical activity was assayed by the method of Wongklom [14]. The 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt was stable radical which solution was green. When added extract, the extract will inhibit ABTS radicals was lighten the color of the solution. ABTS cation radical was generated by reacting 7.40 mM ABTS with 2.45 mM potassium persulfate (1 : 1, v v⁻¹) and leaving in the dark at room temperature for 12 – 16 hours. Then that was diluted to give an absorbance of 1.00 ± 0.20 at 734 nm. The Ya-Nang leaf powder water extract and ascorbic acid standard solution (1 mL) were mixed into 5 mL diluted ABTS⁺ solution and lifted in the dark for 60 min. The absorbance was measured at 734 nm. Results were calculated as milligrams of ascorbic acid equivalents per gram of powder (mg AAE g⁻¹ powder).

Total antioxidant capacity assay

The total antioxidant capacity assay base on the reduction of Mo (VI) to Mo (V) and subsequent formation of a green

phosphate/Mo(V) complex in acid pH with modified by Chahmi *et al.* [15]. Briefly, 0.30 mL of the extract and ascorbic standard solution were mixed 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 mixture was incubated at 95 °C for 90 min and then cooled at room temperature. The absorbance was measured at 695 nm. The total antioxidant capacity was 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 modified by Liu *et al.* [16]. The reduction reaction of complexes Fe³⁺(CN)₆ to Fe²⁺(CN)₆, which makes the solution a darker blue is measured. A 1.2 mL of the Ya-Nang leaf powder water extract was mixed into 1.2 mL sodium phosphate buffer (0.20 M, pH 6.60) and 1.2 mL 1% (w v⁻¹) K₃Fe(CN)₆, and incubated at 50 °C for 20 min. After adding 1.2-mL 10% (w v⁻¹) trichloroacetic acid, the mixture was centrifuged at 3700xg for 10 min. Briefly, the 2 mL supernatant was mixed into 2 mL distilled water and 1 mL 0.10% (w v⁻¹) FeCl₃. The absorbance was measured at 700 nm. The reducing power was expressed as milligrams of ascorbic acid equivalents per gram of powder (mg AAE g⁻¹ powder).

3. Results and Discussion

Total phenolics and flavonoids content

The water soluble phenolics and flavonoids content in the Ya-Nang leaf powder by the different drying methods were shown in Table 1. The highest of the water soluble phenolics and flavonoids content existed in the hot air dried Ya-Nang leaf powder at 60 °C for 5 h (11.277 ± 0.158 mg GAE g⁻¹ powder, 9.485 ± 0.362 mg CE g⁻¹ powder) among the hot air dried at 50 °C for 6 h (11.122 ± 0.280 mg GAE g⁻¹ powder, 9.015 ± 0.190 mg CE g⁻¹ powder), sun dried for 18 h (9.937 ± 0.163 mg GAE g⁻¹ powder, 7.455 ± 0.237 mg CE g⁻¹ powder) and hot air dried powder at 70 °C for 4 h (7.044 ± 0.160 mg GAE

g^{-1} powder, $4.082 \pm 0.182 \text{ mg CE g}^{-1}$ powder). Total phenolics and flavonoids content remained in the hot air dried powder more than the sun dried powder due to the sun drying used a long time for drying and exposed to UV light directly, so these active components may evaporate during drying process [13 – 14]. The hot air drying with highly temperature at 70°C caused the soluble phenolics and flavonoids evaporated and decomposed during drying

process. The process of hot air drying requires higher temperature and increased airflow, which are needed to promote water evaporation and reduce the relative humidity, and finally results in the deterioration of phenolics and flavonoids in raw materials. Some antioxidants will decompose through the mechanism of hydrolysis of esters or glycosides [17 – 18].

Table 1 Total phenolics and flavonoids content of the Ya-Nang leaf powder from the different drying methods

Drying method	Total phenolics content (mg GAE g^{-1} powder ; n = 5)	Total flavonoids content (mg CE g^{-1} powder ; n = 5)
sun drying 18 h	9.937 ± 0.163	7.455 ± 0.237
hot air drying 50°C 6 h	11.122 ± 0.280	9.015 ± 0.190
hot air drying 60°C 5 h	11.277 ± 0.158	9.485 ± 0.362
hot air drying 70°C 4 h	7.044 ± 0.160	4.082 ± 0.182

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

The DPPH free radical scavenging activity, ABTS free radical scavenging activity, total antioxidant capacity and reducing power of the Ya-Nang leaf powder from three different drying methods were showed in Table 2. The highest of DPPH free radical scavenging activity, ABTS free radical scavenging activity, total antioxidant capacity and reducing power existed in the hot air dried powder (60°C 5 h) among the hot air dried (50°C 6 h), sun dried (18 h) and the hot air dried powder (70°C 4 h). The Ya-Nang leaf powder, hot air dried at 60°C for 5 h had DPPH free radical scavenging activity existed $23.293 \pm 0.352 \text{ mg AAE g}^{-1}$ powder, ABTS free radical scavenging activity existed $10.155 \pm 0.393 \text{ mg AAE g}^{-1}$ powder, total antioxidant capacity existed $32.382 \pm 0.414 \text{ mg AAE g}^{-1}$ powder and reducing power existed $6.037 \pm 0.039 \text{ mg AAE g}^{-1}$ powder. The DPPH free radical scavenging activity, ABTS free radical scavenging activity, total antioxidant capacity and reducing power related to total phenolics, flavonoids and phytochemicals content

because these antioxidants can give free electrons or hydrogen radical in the reduction reaction [14]. The results were corresponded by Wongklom found the leaf powder that has been dried by shade drying ($30 \pm 2^\circ\text{C}$) for 24 h as long-term drying, total phenolics and flavonoids evaporated along with water and exposed an UV light during drying process, caused the amount of these components decreased. As a result, the DPPH free radical scavenging activity, ABTS free radical scavenging activity, total antioxidant capacity and reducing power were reduced as well. An increase in the free radical inhibition caused by antioxidant due to 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 leaf powder [18]. It can be deduced that drying methods (drying temperature and time) had an effect on the antioxidant activities of the powder. Wongklom (2023) found that drying temperature and time were affected on the total phenolics, flavonoids, phytochemical contents, and antioxidant activities of the gurmar leaf powder. The hot air dried (50°C 3 h) gurmar

leaf powder had total phenolics, flavonoids, and antioxidant activities were higher than the hot air dried (40 °C 5 h, 60 °C 3 h) and shade dried powder, respectively. [19]. The Ya-Nang leaves contain the antioxidants such as beta-carotene, xanthophyll, vitamin C, vitamin E, tannins and phenolic compounds. In addition, the Ya-Nang leaf powder contains active components such as polysaccharides, polyphenols, and isoquinoline alkaloids, result the powder had DPPH free radical scavenging activity, ABTS free radical scavenging activity, total antioxidant capacity and reducing power. The drying methods take different drying time and temperature can remove the water content from herb. The moisture content is lower than 10%, microorganisms or fungi are not developed in the herbal leaves and powder [10, 18, 19]. The active substances contents in the Ya-Nang powder led to produce in high quality of tea product and others.

The Ya-Nang leaf powder from these drying methods has various medicinal properties such as anti-fever, anti-alcohol poisoning, anti-malaria, antioxidant, anti-inflammation and lowering blood sugar levels [1 – 4]. The active ingredients from the Ya-Nang leaf powder contain high amount of total phenolics and flavonoids such as gallic acid and quercetin contents which can reduce lower blood glucose in induced diabetes mellitus by inhibiting alpha-glucosidase activity. It inhibits the digestion of carbohydrates, reduces glucose absorption in the small intestine, increase the response of cells to insulin and glucose tolerance [3, 4]. Beside, reported to induce insulin secretion and protect the pancreatic beta cell damage in diabetic induced rats [4]. The active substances content in the Ya-nang leaf powder led to produce in the high quality of the Ya-Nang leaf and powder tea products.

Table 2 DPPH free radical scavenging activity, ABTS free radical scavenging activity, total antioxidant capacity and reducing power of the Ya-Nang leaf powder from three different drying methods

drying method	DPPH free radical scavenging activity	ABTS free radical scavenging activity	total antioxidant capacity	reducing power
			mg AAE g ⁻¹ powder; n = 5	
sun drying 18 h	20.698 ± 0.324	9.291 ± 0.407	20.786 ± 0.476	5.137 ± 0.039
hot air drying 50 °C 6 h	22.296 ± 0.660	9.775 ± 0.277	25.733 ± 0.983	5.193 ± 0.212
hot air drying 60 °C 5 h	23.293 ± 0.352	10.155 ± 0.393	32.382 ± 0.414	6.037 ± 0.039
hot air drying 70 °C 4 h	11.924 ± 0.517	8.241 ± 0.300	15.573 ± 0.382	4.326 ± 0.047

4. Conclusion

The temperature and time for drying methods included sun drying, hot air drying at 50 °C for 6 h, hot air drying at 60 °C for 5 h and hot air drying at 70 °C for 4 h were affected on total phenolics, flavonoids and antioxidant activities of the Ya-Nang leaf powder. DPPH free radical scavenging activity, ABTS free radical scavenging activity, total antioxidant capacity and reducing power of the Ya-Nang leaf powder

from three different drying methods related to total phenolics and flavonoids contents. The hot air dried of the Ya-Nang leaf powder at 60 °C for 5 h contains total phenolics (11.277 ± 0.158 mg GAE g⁻¹ powder), total flavonoids (9.485 ± 0.362 mg CE g⁻¹ powder), DPPH free radical scavenging activity (23.293 ± 0.352 mg AAE g⁻¹ powder), ABTS free radical scavenging activity (10.155 ± 0.393 mg AAE g⁻¹ powder), total antioxidant capacity (32.382 ± 0.414 mg AAE g⁻¹ powder) and reducing

power (6.037 ± 0.039 mg AAE g⁻¹ powder) was higher than that of the Ya-Nang leaf powder treated by sun drying, hot air drying at 50 °C for 6 h and hot air drying at 70 °C for 4 h, respectively. The Ya-Nang leaf powder from three different drying methods has lower moisture content and water activity than 10% and 0.6, respectively. The microorganisms did not grow in the leaf powder led to the storage shelf life long time. The results indicated the appropriate drying method at 60 °C for 5 h made the Ya-Nang leaf powder contain the high bioactive compounds and antioxidant activities. It can be used for further development of the commercial Ya-Nang tea products.

5. Acknowledgement

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6. References

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