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Total Polyphenol Content and Antioxidant Activity of The Extracts from *Thunbergia laurifolia* Lindl. Leaves

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Thunbergia laurifolia Lindl. is commonly used as a medicinal herb and a tea in Thailand. In this study, water and various ratios (25, 50, 75 and 100%) of ethanol in water were used as a solvent in extraction of *T. laurifolia*. The total phenolic, flavonoid, tannin contents and antioxidant activity of the *T. laurifolia* leaves extracts were investigated. The extract obtained by 75% ethanol showed the highest total phenolic content (97.80 mg gallic acid equivalent/g crude extract) and the highest total tannin content (11.81 mg tannic acid equivalent/g crude extract). The same extract also exhibited the highest DPPH radical scavenging activity. The 100% ethanol extract showed the highest total flavonoid content (4.20 mg quercetin equivalent/g crude extract). These results indicate that *T. laurifolia* leaves may be considered a source of phytochemicals with important antioxidant properties.

Keywords: *Thubergia laurifolia*, Antioxidant activity, Solvent, Extraction.

Introduction

An antioxidant is a molecule capable of reducing the damage of body cells caused by oxidation by inhibiting the oxidation of other molecules. In the food industry, the synthetic antioxidants (butylated hydroxytoluene BHT; butylated hydroxyanisole BHA; propyl gallate PG and tertiary butyl hydroquinone TBHQ) have been the most widely used. However, the use of synthetic antioxidants regularly showed their toxicity and cause negative effects on human health. Therefore, the search for natural antioxidants of plant origin, have increased significantly in recent years. Natural antioxidants from plant material will be favored in the food industry. Thunbergia laurifolia Lindl. also known as laurel clock vine is a popular ornamental plant commonly found in tropical gardens (Boonyarikpunchai *et al.*, 2014). *T. laurifolia* has been used as medicine for centuries in Thai culture such as cure fever, mild poisons, and

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hangover. Some studies indicate that in the T. laurifolia contain a high amount of antioxidants (Chan *et al.*, 2012; Junsi and Siripongvutikorn, 2016). Extraction yields and bio-activity effect not only depend on the extraction method but also depend on the solvent used for extraction. The dependence of extraction yield on the extraction solvent system is accepted by many other authors (Goli *et al*., 2004; Li *et al.*, 2009). The most suitable solvents are aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate (Sun and Ho, 2005). Ethanol has been known as a good solvent for polyphenol extraction and is safe for human.

In this study extracts were obtained from dried leaf of T laurifolia using different solvent systems (ethanol in distilled water). An extraction was determined by measuring total phenolic, flavonoid, tannin and antioxidant activity (scavenging effect of 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical, ferric reducing power and metal chelating activity).

Materials and methods

Preparation of plant extraction

The mature and healthy leaves of T. laurifolia were harvested from the plant growing in experimental field at King Mongkut's Institute of Technology Ladkrabang and the leaves were cleaned from soil immediately by running tap water, dried-up in a hot-air oven at 45oC for 3 days and ground to small pieces using an electrical blender. Twenty gram of *T. laurifolia* dried leaves was soaked in each 180 ml of different solvents system (25, 50, 75 and 100% ethanol in distilled water) at room temperature, excepted distilled water treatment was placed at 8 ℃ for 3 days. After 3 days, the solutions were filtered through 2 layers of cheesecloth and re-filtered through Whatman no. 93 filter paper. Following filtration, the solutions were dried up by a rotary evaporator (Buchi R215, Switzerland), under a partial vacuum at 45^{o}C until constant crude extract weight was reached. After that each residue was re-extracted 2 times with the same extraction solvent as the same condition of the first extraction procedure.

Determination of total polyphenol content

Determination of total phenolic content

The total phenolic content was quantified using Folin-Ciocalteu method according to the procedures of Chumyam *et al*. (2013). One ml of extract (at concentration 0.25, 0.5 and 1 mg/ml) was added to 4.5 ml distilled water, 0.5 ml of 2N Folin-Ciocalteu reagent. The reaction mixture was vortexed for 5

second and added 4 ml of sodium carbonate (7.5%). Subsequently, the mixture was vortexed for 15 second and incubated for 60 minutes at room temperature in the dark. The mixture was centrifuged at 6000 rpm for 5 minutes at 25^{o}C. The absorbance was read at 765 nm with the spectrophotometer. The experiment was replicated three times in a completely randomized design (CRD). The total phenolic contents were calculated on the basis of the calibration curve of gallic acid standard. Results were expressed as mg gallic acid equivalent per gram crude extract (mg GAE/g CE) of the plant material.

Determination of total flavonoid content

The total flavonoid content of extracts were investigated using the aluminum chloride colorimetry method described by Patel *et al.* (2010) with slight modifications. 0.5 ml of the extract (at concentration 1.0, 2.0 and 4.0 mg/ml) was diluted with 1.5 ml methanol. Then, the extract was added to 0.1 ml of Alumium Chloride (10%), 0.1 ml of 1M Potassium acetate and 2.8 ml distilled water. The mixture was kept at room temperature for 30 minutes. The maximum absorbance of the mixture was measured at 415 nm using a spectrophotometer. The experiment was replicated three times in a completely randomized design (CRD). The total flavonoid content was expressed as milligram quercetin equivalent per gram crude extract (mg QE/g CE).

Determination of total tannin content

The tannins was determined by Folin - method according to the procedures of Tamilselvi *et al.* (2012). 0.1 ml of the extracts (at concentration 2.5, 5.0, 10 mg/ml) was added with 7.5 ml of distilled water and adds 0.5 ml of Folin Phenol reagent, 1 ml of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well, kept at room temperature for 30 min and absorbance was measured at 725 nm. Blank was prepared with water instead of the sample. The experiment was replicated three times in a completely randomized design (CRD). A set of standard solutions of tannic acid is treated in the same manner as described earlier and read against a blank. The results of tannins were expressed in terms of tannic acid mg/g of crude extract.

Determination of antioxidant activity

DPPH radical scavenging activity

The antioxidant activity of the extract was measured by bleaching a purple the reducing ability of antioxidant toward the DPPH radical. The method described by Ebrahimzadeh *et al.*(2008) was used with slight modification. An

aliquot, 2 ml of the extracts of different concentrations were mixed with 2 ml of DPPH solution (100 µM) in ethanol. After incubation in the dark at room temperature for 30 minutes, changed in the absorbance of the extract was measured at 517 nm using a spectrophotometer. Ascorbic acid and butylated hydroxyl toluene were used as standard. Samples were analyzed in triplicate. The percentage inhibition of radicals was calculated using the following formula:

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

Where $A_{control}$ is the absorbance of DPPH solution without extract and A_{sample} is the absorbance of sample with DPPH solution. The half-maximal inhibitory concentration (IC_{50}) was reported as the amount of antioxidant required to decrease the initial DPPH concentration by 50%

Reducing power ability

The method described by Chu *et al.* (2000) was applied in this study to determine the reducing power of the extract. This reducing was investigated by observing the transformation of Fe^{3+} to Fe^{2+}. The extract was diluted with distilled water. The diluted extract (0.5 ml) was mixed with phosphate buffer (2.5 ml, pH 6.6) and potassium ferricyanide (2.5 ml, 1% w/v) in a test tube, followed by incubating in a water bath at 50^{o}C for 20 minutes. After the tube was removed from the water bath, trichloroacetic acid (2.5 ml, 10% w/v) was added into the tube and centrifuged (3000 rpm, 25^{o}C, 10 minutes). The supernatant (2.5 ml) was diluted with distilled water (2.5 ml), and freshly prepared ferric choloride (0.5 ml, 0.1w/v) was added. The mixture was mixed thoroughly and its absorbance was measured at 700 nm using a spectrophotometer.

Metal chelating activity

The chelating ability of the plant extract for ferrous ions (Fe^{2+}) was quantified according to the modified method of Dinis *et al.* (1994). An aliquot, 1 mL of sample extract at various concentrations was mixed with 1.5 ml of ethanol and 50 µL of ferrous sulfate heptahydrate (2 mM) was added. The reaction was initiated by the addition of 100 µL of ferrozine solution (5 mM). Then, the mixture was shaken vigorously and incubated at room temperature for 10 minutes. Absorbance of the sample was them measured at 562 nm. Ethylene-diaminetetra acetic acid was used as standard. The ability in chelating ferrous ions of the sample was expressed as the percentage of metal chelating activity and was calculated using the following equation:

$$\text{Metal chelating effect (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the tested extract. Metal chelating activity is presented by IC_{50} values where concentration providing 50% inhibition of chelate ferrous ions. All tests were performed at least in triplicate, and graphs were plotted using the average of three determinations.

Results

Determination of total polyphenol content

Total phenolic content *(*TPC*)*

Table 1 shows the TPC of the extracts measured using Folin - Ciocalteu method. Results of these assays, demonstrated significant variability in total phenolic content ($P<0.05$). The TPC values of the extract range from 48.47 mg GAE/g CE for water extract to 97.80 mg GAE/g CE for 75% ethanol extract and they decrease in the following order: 75% ethanol > 50% ethanol > 25% ethanol > 100% ethanol > water. Junsi *et al.* (2017) also reported that TPC in crude, dry extract of *T. laurifolia* leaves was 106 mg GAE/g crude, dry extract. However, in the case of Oonsivilai (2006), who reported that aqueous extract of *T. laurifolia* leaves had higher TPC compared to ethanol and acetone extract, these differences in phenolic content might be due to variable factors such as planting location, extraction preparation and stage of leaf development.

Total flavonoid content *(*TFC*)*

Total flavonoid contents of different extracts are given in Table 1. The TFC can be grouped into three levels. The highest level with the highest value belongs to the 100% ethanol extract (4.20 mg QE/g CE). The second level (2.26-2.39 mg QE/g CE) includes the 50% ethanol extract (2.26 mg QE/g CE) and the 75% ethanol extract (2.39 mg QE/g CE). The final level with the lowest TFC (1.33-1.58 mg QE/g CE) includes the water extract (1.33 mg QE/g CE) and the 25% ethanol extract (1.58 mg QE/g CE). This finding was in an agreement with the study of Rojsanga *et al.* (2012) who reported the presence of flavonoid compounds in *T. laurifolia.*

Total tannin content

The recovery of tannin content was shown in Table 1. The tannin content ranged from 7.30 mg TAE/g CE to 11.81 mg TAE/g CE. The highest tannin content belong the 50% ethanol extract (11.42 mg TAE/g CE) but no different

(P<0.05) was observed between 75% ethanol extract (11.81 mg TAE/g CE). Followed by the 25% ethanol extract (10.66 mg TAE/g CE) and the lowest tannin content are the water extract (7.69 mg TAE/g CE) and the 100% ethanol extract (7.30 mg TAE/g CE). No results have been published on the tannin composition of *T. laurifolia.*

Table 1. Contents of total phenolic, flavonoid and tannin in *T. laurifolia* extracts

Solvents (% ethanol)	Total phenolic (mg GAE/g CE)	Flavonoids (mg QE/g CE)	Tannins (mg TAE/g CE)
Water	48.47 ±0.67 [e]	1.33 ±0.03 [c]	7.69 ±0.70 [c]
25	71.66 ±1.33 [c]	1.58 ±0.03 [c]	10.66 ±0.39 [b]
50	88.23 ±2.00 [b]	2.26 ±0.11 [b]	11.42 ±0.33 [a]
75	97.80 ±1.11 [a]	2.39 ±0.07 [b]	11.81 ±0.38 [a]
100	63.03 ±1.27 [d]	4.20 ±0.34 [a]	7.30 ±0.25 [c]

[1]/ [a-e] Means sharing different letters in same column are significant difference (P<0.05)
[2]/ All values were expressed as mean ± standard deviation

Determination of antioxidant activity

DPPH radical scavenging activity

Solvents used for extraction had significant effects on DPPH scavenging capacity determination for *T. laurifolia* (Table 2). The results are expressed as IC_{50} values. The IC_{50} of a compound is inversely related to its antioxidant capacity, as it expresses the amount of antioxidant required to decrease the DPPH concentration by 50%. A lower IC_{50} indicates a higher antioxidant activity of a compound. In this study found that the 75% ethanol extract (IC_{50}: 33.88 mg/l) but no different (P<0.05) was observed between the 50% ethanol extract (IC_{50}: 36.37 mg/l) and possesses the strongest DPPH radical activity. Phenolics were the main antioxidant components, and their total contents were directly proportional to their antioxidant activity. In this study, there is correlation between total phenolic content and DPPH radical scavenging activity.

Reducing power ability

The reducing power of the extract, which may serve as a reflection of its antioxidant activity, was determined using a modified Fe^{3+} to Fe^{2+} reduction assay. Results presented in Table 2 shows that the 50% ethanol extract revealed the highest activity (IC_{50}: 585.58 mg/l) and the water extract possesses the lowest activity (IC_{50}: 1020.93 mg/l) in reducing power.

Metal chelating activity

The metal chelating activity of the crude extracts from *T. laurifolia* were estimated by assessment of their ability to compete with ferrozine for ferrous ions. The recovery of the metal chelating activity of the crude extracts from *T. laurifolia* is expressed in Table 2. The results were showns that the water extract revealed the highest activity in metal chelating (IC_{50}: 2024.96 mg/l). Followed by the 25% ethanol (IC_{50}: 3956.37 mg/l), which had no significant difference ($P<0.05$) with the 50% ethanol extract (IC_{50}: 3969.55 mg/l). The 75% ethanol extract and 100% ethanol extract showed no activity in metal chelating. No results have been published on the metal chelating activity of *T. laurifolia.*

Table 2. Effect of various crude extracts from *T. laurifolia* leaves on antioxidant activity

Solvents (% ethanol)	IC_{50} (mg/l)		
	DPPH	Reducing power	Metal chelating
Water	86.69 ± 0.99 [a]	1020.93 ± 6.13 [a]	2024.96 ± 44.15 [b]
25	42.20 ± 0.28 [c]	646.13 ± 4.77 [c]	3956.37 ± 35.30 [a]
50	36.37 ± 0.2 [d]	585.58 ± 3.40 [d]	3969.55 ± 20.11 [a]
75	33.88 ± 1.34 [d]	634.07 ± 5.95 [c]	Na
100	66.88 ± 2.26 [b]	751.15 ± 5.43 [b]	Na

[1]/ [a-d] Means sharing different letters in same column are significant difference ($P<0.05$)
[2]/ Na = No activity
[3]/ All values were expressed as mean ± standard deviation

Conclusion

The present study evaluated polyphenol compounds and antioxidant activity of five different ethanol ratios in water of *T. laurifolia.* The extracts from *T. laurifolia* leaves were found rich polyphenol compounds. Both of the 50% ethanol extract and the 75% ethanol extract showed the best scavenging activity of DPPH radical. The 50% ethanol extract showed the strongest in reducing power ability. Otherwise, metal chelating activity ability is better for the water extract. Therefore, *T. laurifolia* leaves may be considered a source of important phytochemicals with important antioxidant properties.

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