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Article

Selective Entrapment of Pb^{2+} from Fresh *Thunbergia laurifolia* Leaves Extract and *Thunbergia laurifolia* Tea Extract

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The leaves of *Thunbergia laurifolia* and its tea were extracted by water. The abilities to chelate heavy metal ions of the extracts were studied by inductively coupled plasma-optical emission spectroscopy (ICP-OES) and atomic absorption spectroscopy (AAS). The results showed that the extracts exhibited high selectivity for Pb^{2+} chelation via a favourable-selective precipitation to Pb^{2+} in aqueous solutions compared to other metal ions, such as Zn^{2+}, Cu^{2+} and Fe^{3+}. The Pb^{2+} removal capability of the extracts were 51-52%. The selective Pb^{2+}-trapping process of the extracts of *Thunbergia laurifolia* could be attributed to predominant presence of the phytochemical compounds, tannin and saponin, in the *Thunbergia laurifolia* leave and tea. Moreover, *Thunbergia laurifolia* tea also exhibited antioxidant property as demonstrating by the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical method.

Keywords: Pb^{2+}-selectivity, Pb^{2+}-screening, natural and aqueous-based *Thunbergia laurifolia* extracts

Introduction

Lead ion (Pb^{2+}) as a toxic heavy metal causes the contamination of the water environment. Exposure to Pb^{2+} can cause learning disorders, impair of cognitive functions, acute pain such as vomit, constipation, and severe abdominal pain.[1,2] Moreover, exposure to high dose of lead can cause paralysis, convulsions, delirium, coma, or death.[2] The sources of Pb^{2+} contaminant in an environment are from both natural occurrence and human activities such as mineral mine, electronic device manufacturing, and waste from battery factory.[3,4] Lead poison can be cured by taking chelating agents such as D-penicillamine or Ca_2Na_2EDTA. However, some side effects of taking ethylenediamine tetraacetic acid (EDTA), including hives, fever, and high blood pressure were reported. Additionally, EDTA can bind to the life-essential metal ions such as Ca^{2+}, Cu^{2+}, Fe^{2+} and Zn^{2+}. Therefore, therapeutic of lead poisoning by using the natural product which will not bind to those essential metal ions is an alternative option.

Thunbergia laurifolia ("Rang Jued" in Thai) is a local Thai plant which has been commonly used in a traditional Thai medicine.[5] It has been used to heal pesticide poisoning, rat poisoning, and alcohol poisoning.[6,7] To the best of our knowledge, only few studies of Pb^{2+} detoxification by using *Thunbergia laurifolia* extract have been reported. Palipoch *et al.*[8] reported that 50% ethanol (EtOH) extraction of *Thunbergia laurifolia* could reduce the toxic from lead poison. Herein, we demonstrated Pb^{2+} ion entrapment efficiency by the extracts from both collected fresh *Thunbergia laurifolia* leaves and tea from collected dried *Thunbergia laurifolia* which were easy to prepare and cost-effective. And the Pb^{2+} ion entrapment efficiency was compared to other metal ions including Cu^{2+}, Zn^{2+} and Fe^{2+}. In addition, the phytochemical compounds, which were essential for the Pb^{2+} ion trapping process, were determined. Furthermore, the antioxidant property of *Thunbergia laurifolia* extract was also investigated by the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical method.

Experimental

Chemical

Metal chlorides and metal nitrates were used in the metal ions entrapment studied. DPPH analytical grade was purchased from Sigma-Aldrich (St. Louis, USA). All chemicals were used as received.

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Preparation of fresh *Thunbergia laurifolia* extract

The collected *Thunbergia laurifolia* leaves were washed 5 times by deionized water and were left to dry at room temperature. Five grams of the cleaned leaves were grinded with addition of 45 mL of deionized water. Then the aqueous solution was separated from the mixture by a chess cloth and was filtered under a vacuum. Finally, the 0.10 g mL^{-1} fresh *Thunbergia laurifolia* extract aqueous solution was obtained by diluting the filtrate to 50.00 mL with deionized water.

Preparation of *Thunbergia laurifolia* tea extract

The collected *Thunbergia laurifolia* leaves were washed several times with deionized water and then were dried under sun light until the mass was constant to obtain a *Thunbergia laurifolia* tea. Five grams of the tea was grinded with addition of 45 mL of deionized water. Then the aqueous solution was separated from the mixture by a chess cloth and was filtered under a vacuum. Finally, the 0.10 g mL^{-1} *Thunbergia laurifolia* tea extract aqueous solution was obtained by diluting the filtrate to 50.00 mL with deionized water.

Preparation of metal ion solutions

All metal salts (Pb^{2+}, Zn^{2+}, Cu^{2+} and Fe^{2+}) were separately dissolved and diluted by deionized water. The final concentrations of all ions were adjusted to 1.00×10^{-2} M.

Metal ions entrapment by fresh *Thunbergia laurifolia* extract

To study the metal ions entrapment efficiency, fresh *Thunbergia laurifolia* extract and metal ions were mixed and vortexed for 2 min with the ratios as shown in Table 1. The ratio of each extract was chosen based on the ratio that allowed clear observation of the precipitation (entrapment) of the extract. Then the supernatant was separated by centrifugation and filtration through a 0.45 μm Nylon® syringe filter. The concentration of unbinding metal ion was determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES).

Metal ions entrapment by *Thunbergia laurifolia* tea extract

To study the metal ions entrapment efficiency, *Thunbergia laurifolia* tea extract and metal ions were mixed and vortexed for 2 min with the ratios as shown in Table 2. Then the supernatant was separated by centrifugation and filtration through a 0.45 μm Nylon® syringe filter before 50 times dilution. The concentration of unbinding metal ion was determined by AAS.

Table 1. The volume contents of metal ions solution and fresh *Thunbergia laurifolia* extract solution for entrapment study

Solutions	Volume / mL
1.00×10^{-2} M metal ions	0.20
Deionized water	13.80
0.10 g mL^{-1} fresh *Thunbergia laurifolia* extract	6.00
Total volume	20.00

Table 2. The volume contents of metal ions solution and *Thunbergia laurifolia* tea extract solution for entrapment study

Solutions	Volume / mL
1.00×10^{-2} M metal ions	2.00
0.10 g mL^{-1} *Thunbergia laurifolia* tea extract	2.00
Total volume	4.00

Phytochemical screening of *Thunbergia laurifolia*

The phytochemical compounds in *Thunbergia laurifolia* were expected to involve the metal ions trapping process,[9] hence types of phytochemical compounds in both fresh *Thunbergia laurifolia* and *Thunbergia laurifolia* tea were investigated using standard methods with some modifications.[10] In this work, flavonoid, tannin, saponin, anthraquinone, terpenoid and cardiac glycoside were monitored.

Test for flavonoid

200 mg of *Thunbergia laurifolia* was suspended by 5 mL of EtOH:H_2O (1:1) solution in the test tube. Three pieces of magnesium ribbons were added to suspension before heating up to 70 ºC. Then 2-3 drops of concentrated HCl were added to the mixture. If flavonoid is presence in *Thunbergia laurifolia*, the color of solution will change to yellow, orange, or red.

Test for tannin

200 mg of *Thunbergia laurifolia* was added to 5 mL of deionized water and was then warmed in water bath. The resulted solution was collected by filtering and 2-3 drops of 0.01 M $FeCl_3$ solution was then added into the solution. If tannin exists, the dark green or dark blue color shall be observed.

Test for saponin

200 mg of *Thunbergia laurifolia* was added to 5 mL deionized water and the mixture was then boiled. The resulted solution was collected by filtration and 3 mL of deionized water was then added into the solution. After that, the solution was vigorously shaken. The appearance of bubble in solution is an evidence for the presence of saponin.

Test for anthraquinone

200 mg of *Thunbergia laurifolia* was added to 10 mL of 10% H_2SO_4, the mixture was then warmed in water bath for 5 min. Next, the resulted solution was collected by filtration and cooled down to room temperature. Then anthraquinone was extracted by chloroform. After that, a few drops of 10% NH_3 solution was added into the organic filtrate. The existence of anthraquinone will lead to appearance of pink solution.

Test for terpenoid

5 mL of petroleum ether was added to 0.20 g of *Thunbergia laurifolia* in order to extract terpenoid. 2 mL of chloroform was then added into solution followed by addition of 3 mL of concentrated H_2SO_4. Brown solution will be observed if there is presence of terpenoid.

Cardiac glycoside determination

Cardiac glycoside was extracted by mixing 0.20 g of *Thunbergia laurifolia* and 3 mL of petroleum ether. The petroleum ether phase was then separated and was dissolved in 80% EtOH. The presence of cardiac glycoside was determined by Liebermann test. Briefly, 3 drops of glacial acetic acid and conc. H_2SO_4 were added into the petroleum ether solution. The obtained blue or greenish blue solution was an evidence for the presence of cardiac glycoside.

Antioxidant property of *Thunbergia laurifolia* tea extract

The reduction of DPPH free radical was a common method to investigate the antioxidant activity. Thus, the antioxidant activities of the *Thunbergia laurifolia* tea extracts were conducted by the DPPH free radical method with some modifications.[10-12] The following modified DPPH free radical method was applied for antioxidant activity test in this work.

Antioxidant test in solvent

The standard DPPH was prepared by dissolved 2.40 mg of DPPH in methanol with final volume of 10 mL. The DPPH solution (0.5 mL) was then mixed by 2 mL of deionized water to obtain standard DPPH solution. In order to study the antioxidant activity, various concentration of *Thunbergia laurifolia* tea extract (0.1000, 0.0100 and 0.0010 g mL^{-1}) were mixed by 0.5 mL of standard DPPH solution and the changes of solution color were monitored. The violet color of the DPPH solution disappears, and the solution become colorless in the presence of the antioxidant molecules.

Antioxidant test on thin-layer chromatography (TLC)

The standard DPPH solution was spotted on thin-layer chromatography (TLC) plate and was dried. *Thunbergia laurifolia* tea extracts with various concentration (0.1000, 0.0100 and 0.0010 g mL^{-1}) were then dropped on DPPH spot and the change of spot colors were observed.

Results and Discussion

Metal ions entrapment efficiency of fresh *Thunbergia laurifolia* leaves extract

The entrapment results from direct mixing of metal ions solution with fresh *Thunbergia laurifolia* leaves extract (30 mg mL^{-1}) were summarized in Table 3 and Figure 1. It could be noticed that fresh *Thunbergia laurifolia* leaves extract could entrap Pb^{2+} with significantly higher percentage than other metal ions including Zn^{2+}, Cu^{2+}, and Fe^{2+}. Entrapment of Pb^{2+} up to 51.44 % was observed (initial Pb^{2+} concentration is 21.23 mg L^{-1}). This result indicated that the selectivity of the fresh *Thunbergia laurifolia* extract toward Pb^{2+} was superior than Zn^{2+}, Cu^{2+}, and Fe^{2+}.

Table 3. The entrapment of metal ions by fresh *Thunbergia laurifolia* leaves extract

Metal ions	Concentration / (mg L^{-1})			Percentage entrapment / %
	Before addition of *Thunbergia laurifolia* leaves extract	After addition of *Thunbergia laurifolia* leaves extract	Entrapped metal ions	
Pb^{2+}	21.23	10.31	10.92	51.44
Zn^{2+}	4.41	3.67	0.74	16.78
Cu^{2+}	5.60	5.10	0.50	8.93
Fe^{2+}	1.18	0.98	0.20	16.95

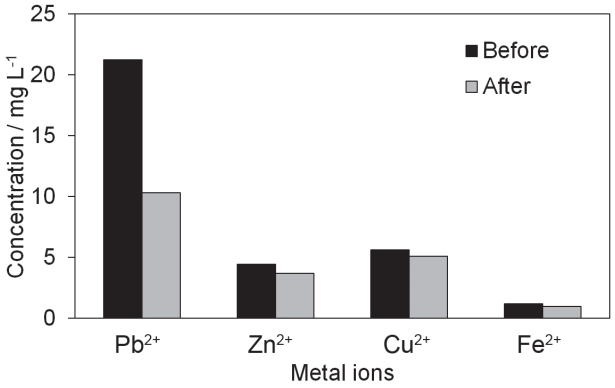

Figure 1. Comparison the metal ions entrapment abilities of fresh *Thunbergia laurifolia* leaves extract before and after mixing.

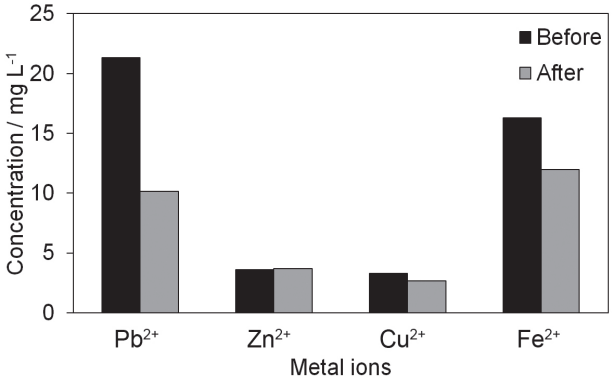

Figure 2. Comparison the metal ions entrapment abilities of *Thunbergia laurifolia* tea extract before and after mixing.

Metal ions entrapment efficiency of *Thunbergia laurifolia* tea extract

The entrapment abilities of *Thunbergia laurifolia* tea extract were summarized in Table 4 and Figure 2. It could be noticed that *Thunbergia laurifolia* tea extract (50 mg mL^{-1}) exhibited similar Pb^{2+} entrapment ability compared to fresh *Thunbergia laurifolia* leaves extract. Pb^{2+} entrapment up to 11.18 mg L^{-1} from initial concentration of 21.32 mg L^{-1} (52.44%). In contrary, Fe^{2+} and Cu^{2+} entrapment abilities were only 26.63% and 19.70%, respectively, while the Zn^{2+} entrapment ability of *Thunbergia laurifolia* tea extract was negligible. The slight increasing of Zn^{2+} concentration could be caused by the spectral interference from Fe and Cu.[13-15] According to the previous research, iron (Fe) and copper (Cu) ions are the essential micronutrients for plants.[16-18] Thus Fe^{2+} could release from *Thunbergia laurifolia* tea extract, which led to increasing of Zn^{2+} concentration. This result suggested that the selectivity of the *Thunbergia laurifolia* tea extract toward Pb^{2+} was significantly greater than Zn^{2+}, Cu^{2+}, and Fe^{2+}.

Table 4. The entrapment of metal ions by *Thunbergia laurifolia* tea extract

Metal ions	Concentration / (mg L^{-1})			Percentage entrapment / %
	Before addition of *Thunbergia laurifolia* tea extract	After addition of *Thunbergia laurifolia* tea extract	Entrapped metal ions	
Pb^{2+}	21.32	10.14	11.18	52.44
Zn^{2+}	3.58	3.68	–0.10	–2.79
Cu^{2+}	3.30	2.65	0.65	19.70
Fe^{2+}	16.30	11.96	4.34	26.63

Phytochemical compound screening

The testing of phytochemical compounds in *Thunbergia laurifolia* was summarized in Table 5. The results show that *Thunbergia laurifolia* extract consist of tannin and saponin as evidenced by the change in color of *Thunbergia laurifolia* extract solution from brown to black color after adding tannin testing reagent and by the bubble appeared in *Thunbergia laurifolia* extract solution after adding saponin testing reagent and vigorously shaken. For the rest phytochemical compounds including flavonoid, anthraquinone, terpenoid and cardiac glycoside, the *Thunbergia laurifolia* extract solution were not changed after adding those testing reagents. This suggested that *Thunbergia laurifolia* did not consist of flavonoid, anthraquinone, terpenoid and cardiac glycoside or it should contain little amount lower than the limit of detection. Although many phytochemicals including phenol, flavonoid, tannin, sterols and cardiac glycoside were found in *Thunbergia laurifolia* extract,[19,20] variations in phytochemical contents between *Thunbergia laurifolia* leaves of different ages, collection times, and locations were also reported.[19] The phytochemical content of *Thunbergia laurifolia* leaves in different studies were shown in the Table 6. Considering the structure of tannin and saponin as shown in Figures 3 and 4, tannin consisted of many hydroxyl groups (–OH) in the form of a catechol group (*o*-dihydroxyphenyl) or galloyl group (trihydroxyphenyl) which are essential to complex with metal ions.[21,22] This hydroxyl groups could bind to Pb^{2+} through Pearson acid

Table 5. The results of phytochemical compounds testing

Tested phytochemicals	Tested results
Flavonoid	not changed
Tannin (phenolic compound)	changed from brown to back colour
Saponin	has bubbled
Anthraquinone	not changed
Terpenoid	not changed
Cardiac glycoside	not changed

base concept (HSAB). The hard base of oxygen atom from tannin could strongly bind with the hard acid or moderate acid such as Pb^{2+}. In addition, previous research also reported the ability of tannin to entrap Pb^{2+}.[23] Briefly, tannin which obtained by aqueous extract of the chestnut shells could trap Pb^{2+} up to 61%. Thus, to confirm the role of tannin to bind with Pb^{2+}, the tannin testing reagent was added to the *Thunbergia laurifolia* extract solution before and after entrapping Pb^{2+} ion. The results in Figure 5 illustrated that the color of *Thunbergia laurifolia* extract solution changed from brown to black after adding tannin testing reagent. In other hand, the solution contains both *Thunbergia laurifolia* extract and Pb^{2+} ion did not change in color after adding tannin testing reagent. It could be due

Table 6. Comparison of phytochemical compounds found in *Thunbergia laurifolia*

	Tannin	Saponin	Flavonoid	Anthraquinone	Terpenoid	Cardiac glycoside	Others
Junsi *et al.*[20]	+	–	+	N.D.	–	+	phenols, sterols
Chan *et al.*[19]			+ (apigenin)		+ (iridoid glucosides, grandifloric acid)		phenolic acids, delphinidin derivative, glycosides
Cock and Kukkonen[24]	+	+	+	–	–	–	
Saeed *et al.*[10]	+	+	+	+	+	+	alkaloids
This work	+	+	–	–	–	–	N.D.

N.D.: not detected; +: presence; –: absence.

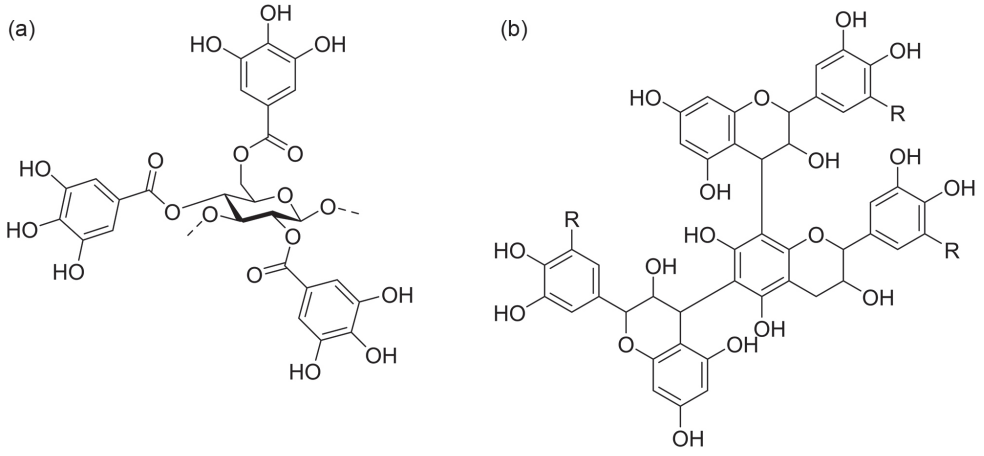

Figure 3. Structure of tannin molecule: (a) hydrolysable tannin (HT) and (b) condensed tannin.

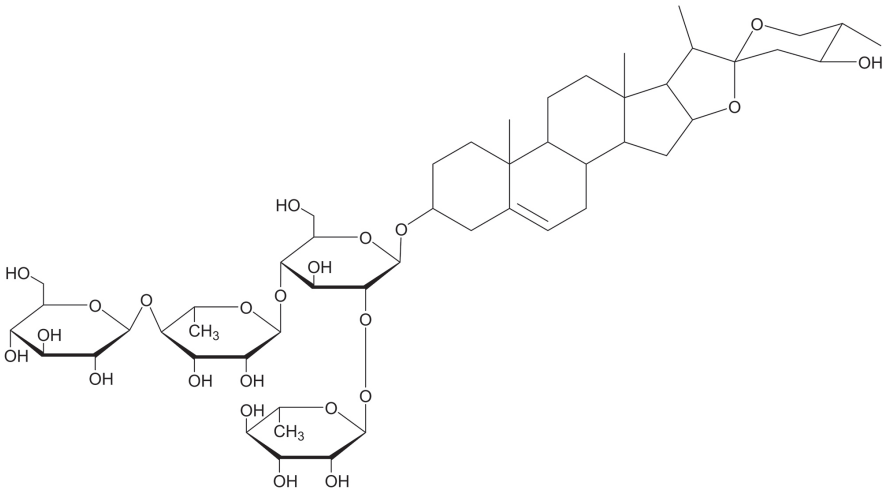

Figure 4. Structure of steroidic saponin.

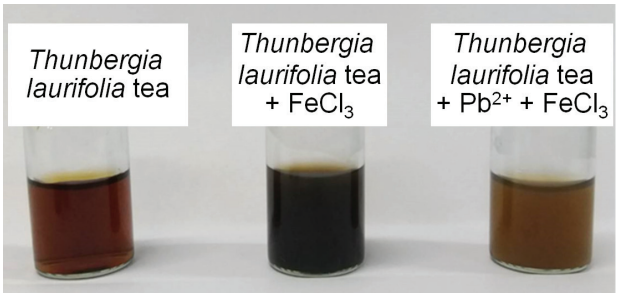

Figure 5. The change of tannin content before and after the Pb^{2+} entrapment.

to that almost of tannin in solution was strongly bound with Pb^{2+} hence tannin cannot react with the testing reagent. This suggested that tannin in *Thunbergia laurifolia* extract involved the entrapment of Pb^{2+} ion.

Antioxidant activity of *Thunbergia laurifolia* tea extract

Mixing of the *Thunbergia laurifolia* tea extract and DPPH solution led to color change from violet to brown which was the color of *Thunbergia laurifolia* tea extract as shown in Figure 6. In fact, *Thunbergia laurifolia* tea extract with concentration that was equal or greater than 0.100 g mL^{-1} led to appearance of brown color solution. The result suggested that the *Thunbergia laurifolia* tea extract contained antioxidant molecules which was in good agreement to the previous study.[6] Briefly, Junsi and Siripongvutikorn[6] compiled the biological activity of the extract of *Thunbergia laurifolia* and they reported that *Thunbergia laurifolia* extract exhibited antioxidant activity which was due to the high total phenolic content.

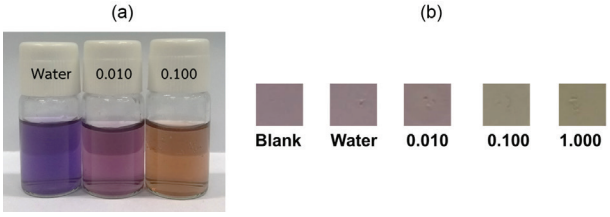

Figure 6. Comparison of the antioxidant activities of *Thunbergia laurifolia* tea extract: (a) in deionized water and (b) on TLC plate.

Conclusions

The fresh *Thunbergia laurifolia* leaves extract and *Thunbergia laurifolia* tea extract exhibited promising ability to trap Pb^{2+} in aqueous solution up to 51.44% and 52.44%, respectively. Importantly, the extracts were highly selective to Pb^{2+} which led to significant lower affinity toward the essential metal ions of human (Zn^{2+} and Fe^{2+}). Several phytochemical compounds in the extracts were investigated by the standard tests. The tests showed that tannin and saponin, which were important for the Pb^{2+} entrapment process, were presence in the extracts of both fresh *Thunbergia laurifolia* leaves and *Thunbergia laurifolia* tea. Moreover, the DPPH assays confirmed that *Thunbergia laurifolia* tea extracts consisted of the antioxidant compounds. Thus, the natural and aqueous-based *Thunbergia laurifolia* extracts were a candidate material for screening Pb^{2+} and reducing the toxic from lead poison.

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