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Preliminary Phytochemical Profile analysis of *Thespesia populnea* (Linn.) Soland ex Correa in Pak Phanang, Nakhon Si Thammarat

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ABSTRACT

Thespesia populnea (Linn.) Soland ex Correa is distributing throughout the tropical region in the world, as in Thailand, they have presented at the swamp region in which freshwater meets seawater. This plant had been reported as a medicinal plant containing flavonoids: quercetin and kaempferol and widely used every part of the plant in traditional medicine treating dysentery, urinary tract problems, so the aim of this study is to a screening of the *T. populnea* phytochemical profile and antioxidant properties of the chemical compound using the thin layer chromatography (TLC) method. In the phytochemical profile of the *T. populnea*, we found alkaloids in leaf, flower, and fruit extracts. The saponin flavonoid in leaf and fruit extracts by using the specific chemical spraying to visualized the reaction on the TLC plate. The antioxidant regarded to the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging reaction on the TLC plate we found two dominant areas in leaf and fruit fingerprint track on the TLC plate: leaf extract area were 436.67±25.12 and 504.33±31.57, and fruit extract area were 300.67±30.61 and 388.67±6.35 at Rf 0.42 and 0.59, respectively. **Conclusion**: we found leaf and fruit of *T. populnea* plant had flavonoid and alkaloid which positive to the DPPH reaction on the TLC plate.

Keywords: *Thespesia populnea*, Portia tree, Phytochemical profile, Anti-oxidant

INTRODUCTION

The Phytochemical profile is generally used to describe the compounds in the plant, which are produced by plants including the secondary metabolites. Plant secondary metabolite is a various compound which plant produced via the plant cell through metabolic pathways derived from the primary metabolic pathways. Plant secondary metabolite was classified according to their chemical structure and possess various biological activities (Hoffmann, 2003; Xu *et al*., 2015). By now, the traditional medicinal herbs would be wildly used to understanding the active compound and chemical composition inside the plant is power helpful to make herbs value (Saeidnia *et al*., 2011; Anbalahan, 2017).

Thespesia populnea (Linn.) Soland ex Correa is classified in the family Malvaceae and is commonly named as Portia tree and Thai common name is Po Tha lay (Warrier, 2010). *T. populnea* is a tropical coastal plant in Southeast Asia, in Thailand, which is commonly found in the eastern and southern parts of Thailand. Parts of the plant are utilized as wood for furniture and others, used as traditional medical treatment, the bark is used for washing skin diseases, leaf extracts are applied on inflamed and swollen joints, flower extracts are anti-hepatotoxic (Shirwaiker *et al*.,1995; Suvarna *et al*., 2018) and anti-steroidogenic activities (Kavimani *et al*., 1999), fruit yellow extract juice used to treat insect bites, gonorrhoea, ringworm, migraine, fistula, sprains, and wart removal, and fruit extracts are used as has wound healing agent (Kavimani *et al*., 1999).

Preliminary phytochemical screening showed the presence of a high amount of phenolics, tannins, and flavonoids in so many plants, which plays the important role in the anti-oxidant activity preventing the generation of free radicals (Qamara *et al*., 2018; Abdelaziz *et al*., 2020; Gebashe *et al*., 2020), so the purpose of this study is to screen the phytochemical profile and chemical compound group by using the specific chemical spraying and the anti-oxidant property of *T. populnea* plant parts based on thin-layer chromatography method.

MATERIAL AND METHODS

Plant collection

Thespesia populnea (Linn.) Soland ex Correa was collected from Pak Phanang district in Nakhon Si Thammarat (Figure 1) which is located at longitude 100°1′4″E and latitude 80°14′2″N. The sample collection was leaves, flowers, and fruits. Samples were then dried at 50°C in the incubator for over two nights and ground into powdery.

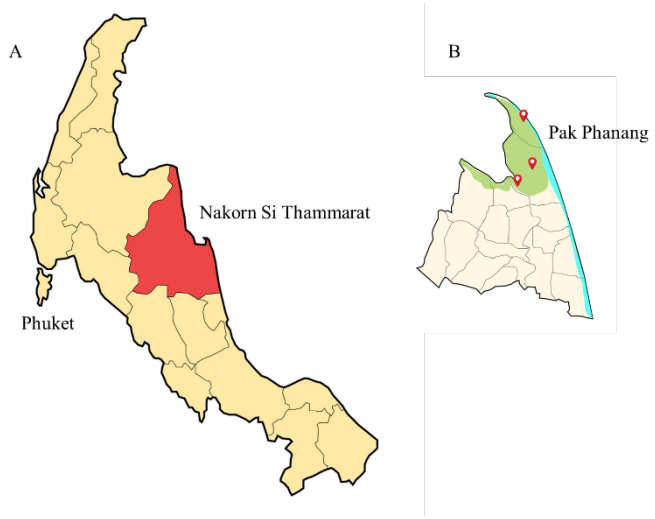

Figure 1. *Thespesia populnea* collection sample site; A: southern part of Thailand, Nakhon Si Thammarat is represented in the red area, B: Pak Phanang District represented in a green area.

Sample extraction

Briefly, 300 mg of *T. populnea* powdery was extracted using a reflux method with 10 ml of methanol at 70°C and shaking at 200 rpm for 2 h. Then, the solvent was removed under vacuum at 70ºC using a rotary evaporator (Buchi, Labortechnik AG, Switzerland). The extract was then resuspended in 1 ml methanol and centrifuged to discard the remaining pellet at 25°C, 10,000 rpm for 5 min.

***Thespesia populnea* fingerprint**

Ten microliters of the extract were spotted onto the 10 x 10 cm TLC plate (TLC plates silica gel 60 F_{254}, MERCK) using the LINOMAT5 (CAMAG) and separated in the saturated developing TLC chamber containing the mobile phase as; toluene: acetonitrile: ethyl acetate: glacial acetic acid (35:5:15:0.15) until the solvent font at 90 mm, removed the TLC plate from the developing TLC chamber and blow-dried. The fingerprint was examined under the white light, 254 and 366 nm luminescent, then documentation.

Preliminary phytochemical methods

Test for Alkaloids. Spray the fingerprint TLC plate with a solution of 37% formaldehyde in a concentration of sulfuric acid (1:10) immediately after taking the plate from the developing chamber.

Test for Saponins. Solution **a**: dissolve 0 . 8 5 g bismuth (III) nitrate in 10 ml glacial acetic acid and 40 ml water. Solution **b**: dissolve 8 g potassium iodide in 20 ml water. Stock solution: Mix equal parts of a and b. The mixture can be stored in a dark bottle for a long time. Spray solution: Mix 1 ml stock solution with 2 ml glacial acetic acid and 10 ml water before use (Munier and Macheboeuf, 1951, Jatzkewitz *et al*., 1953).

Test for Flavonoid. Spray solution: 1 % ethanolic solution of aluminium chloride. Yellow fluorescence in long-wave UV light (Gage *et al*., 1951)

Anti-oxidant property screening on TLC

The dried-fingerprint of *T. populnea* on the TLC plate was then used to spray the DPPH (0.05% 2,2-diphenyl-1-picrylhydrazyl in ethanol) to develop the reaction on the TLC plate by incubated at room temperature in dark for 15 min. The positive reaction was observed as the yellow band on the TLC plate.

Image J analysis on TLC plate

The *T. populnea* fingerprint on the TLC plate was imported to the ImageJ to quantitated the area of the reaction (Schneider *et al*., 2012, Hartig 2013, Rueden *et al*., 2017).

RESULTS

Preliminary phytochemical profile analysis

The plant part of the *T. populnea* extracts as leaves, flowers and fruits could be separated on the silica gel 60 F_{254} TLC plates using the mobile phase as toluene: acetonitrile: ethyl acetate: glacial acetic acid (35:5:15:0.15) and visualized under 254 and 366 nm luminescent (Figure 2A and 2B).

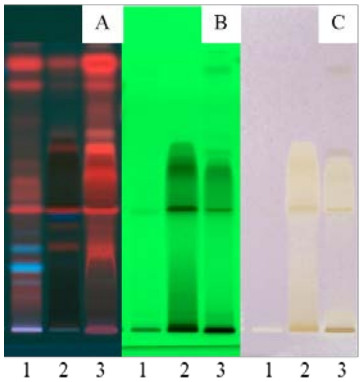

Figure 2. *Thespesia populnea* fingerprint on TLC plate under 366 nm (A), 254 nm (B), and after DPPH spray (C). Track 1: is a flower extract, Track 2: is a leaf extract and Track 3: is a fruit extract.

The separated fingerprint on the TLC plate was post-visualized for alkaloid, saponin, and flavonoid by spraying the specific reagents: in Table 1 we found the alkaloid in all *T. populnea* plant parts, both saponin and flavonoid were found in *T. populnea* leaf and flower extracts (Table 1).

Table 1. Test for preliminary phytochemical profile

Phytochemical profile	Flower extracts	Leaf extracts	Fruit extracts
Alkaloids	+	+	+
Saponins	-	-	-
Flavonoids	-	+	+

Note: +; presence, -; absent

Anti-oxidant reaction

The positive reaction was the presence of the yellowish band on the TLC plate which could be seen under white light (Figure 2C). The reaction of the DPPH to the chemical constituents separated and presented on the *T. populnea* fingerprint TLC plate was analyzed using the ImageJ. Within the track, bands of the DPPH positive (anti-oxidant reaction) were dominant at Rf 0.42 and Rf 0.59 both in leaf and fruit extracts and absence in flower extract (Figure 2C). Leaf extract areas were 436.67±25.12 and 504.33±31.57, and fruit extract areas were 300.67±30.61 and 388.67±6.35 at Rf 0.42 and 0.59, respectively (Table 2).

Table 2. ImageJ area analysis of the positive reaction to DPPH

Phytochemical Profile (Rf)	Flower extracts	Leaf extracts	Fruit extracts
0.42	-	436.67±25.12	300.67±30.61
0.59	-	504.33±31.57	388.67±6.35

DISCUSSION

We found the alkaloids and flavonoids in the phytochemical profile of *T. populnea* leaf, fruit, and flower methanol extracts on the TLC plate. Leaf and fruit extracts were positive to the DPPH assay on the TLC plate which about two times the area of the yellowish of the reaction (Table 3). The two dominant yellow bands on each track (leaf and fruit) might mean the main active compound in the *T. populnea* for anti-oxidant activity (Panchal and Shah, 2017; Panchal *et al*., 2020). However, the phytochemical constituents in *T. populnea* would be further analyzed and identify, especially for the active substance.

CONCLUSION

The *T. populnea* had been found alkaloid in leaf, fruit, and flower methanol extract and flavonoid in leaf and fruit methanol extract. The *T. populnea* fingerprint had positive to the DPPH reaction on the TLC plate by in leaf more than in fruit extract.

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