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PHYTOCHEMICAL SCREENING AND FREE RADICAL SCAVENGING ACTIVITY OF SELECTED THAI MEDICINAL PLANTS

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Abstract: The objectives of this study were to determine the phytochemicals in Thai medicinal plant extracts and to screen the antioxidant activity of the extracts. Thai medicinal plant used in this research were *C. garrettiana, P. merkusii, D. parviflora, T. hoaensis, D. loureiri, T. siamensis, M. elengi, E. antiquorum, P. ribesoides* and *O. indicum.* The phytochemical including reducing sugars, anthraquinones, terpenoids, flavonoids, phenolic compounds, saponins, tannins, alkaloids, cardiac glycosides and proteins were screened. All plant extracts contained terpenoids, flavonoids and phenolic compounds. The antioxidant assays used were DPPH and FRAP. *P. ribesoides* and *D. parviflora* exhibited the most potent antioxidant activity, and this was associated with the data of total phenolic and flavonoid contents those were responsible to the anti-oxidative ability of the extracts. The results revealed the potential uses of the medicinal plant extracts as the dietary supplements as free radical scavenger. Moreover, they could be used as active ingredients in cosmetic formulations.

Keywords: Antioxidant, extract, Free radical scavenging, Phytochemicals, Plant

บทคัดย่อ: งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาพฤกษเคมีในสารสกัดจากสมุนไพรพื้นบ้านของประเทศไทย และเพื่อทดสอบฤทธิ์ต้านออกซิเดชันของสารสกัด พืชตัวอย่างสมุนไพรไทยพื้นบ้านที่นำมาศึกษาได้แก่ แสมสาร, สนสองใบ, สักขี, จันทน์ขาว, จันทน์แดง, ไผ่รวก, ขอนดอก, กะลำพัก, สะค้าน และ เพกา องค์ประกอบทางเคมีในพืชที่ทำการทดสอบ ได้แก่ น้ำตาลรีดิวซิง, แอนทราควิโนน, เทอปีนอยด์, ฟลาโวนอยด์, ฟีนอลิก, ซาโปนิน, แทนนิน, แอลคาลอยด์, คาร์ดิแอคไกลโคไซด์ และโปรตีน จากการศึกษาพบว่าพืชทุกชนิดที่นำมาศึกษามีเทอปีนอยด์, ฟลาโวนอยด์ และ ฟีนอลิก เป็นองค์ประกอบ การทดสอบฤทธิ์ต้านออกซิเดชันของสารสกัดพืชโดยวิธีการ DPPH และ FRAP พบว่า สารสกัดสะค้าน และสักขีมีฤทธิ์ต้านออกซิเดชันสูงที่สุด ข้อมูลที่ได้สอดคล้องกับปริมาณของสารฟีนอลิกและฟลาโวนอยด์ที่วิเคราะห์ได้ในพืชดังกล่าว เนื่องจากสารกลุ่มฟีนอลิกและฟลาโวนอยด์เป็นสารกลุ่มสำคัญที่ออกฤทธิ์ต้านออกซิเดชัน การศึกษานี้แสดงให้เห็นถึงศักยภาพในการนำสารสกัดพืชมาใช้เป็นผลิตภัณฑ์เสริมอาหารเพื่อต้านอนุมูลอิสระ นอกจากนี้ยังสามารถนำมาใช้เป็นสารสำคัญสำหรับผลิตภัณฑ์เครื่องสำอางได้อีกด้วย

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INTRODUCTION

Phytochemicals, naturally occurring in the medicinal plants play a role in defense mechanisms and protection from various diseases. They are primary and secondary compounds. Proteins, chlorophyll, and common sugars are included in primary constituents, and secondary compounds are terpenoids, alkaloids, anthraquinones, and phenolics. Terpenoids, the largest group of phytochemicals, traditionally used as medicinal purposes in

India and China, are currently being explored as anticancer agents in clinical trials (Thoppil and Bishayee, 2011). Alkaloids found in medicinal plants are used as anaesthetic agents. Anthraquinones are reported to reduce inflammation in arthritis patients and also inhibits the growth of cancer cells. Phenolic compounds are phytochemicals which play a major role in the protection of oxidation processes.

Medicinal plants are major source of natural antioxidant products. Plants produce a very interesting antioxidant compounds including carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols and tocotrienols. These antioxidants compounds from plants are believed to have an important role in the maintenance of human health because our endogenous antioxidants provide insufficient protection against the constant and unavoidable challenge of reactive oxygen species (Krishnaiah *et al*., 2007). Oxidative stress enhances pathological processes contributing to cancer, cardiovascular, and neurodegenerative diseases, and dietary antioxidants could counteract these deleterious processes (Qiu *et al*., 2012; Shah *et al*., 2010). The balance between anti-oxidation and oxidation is believed to be critical in maintaining a healthy biological system (Farhan *et al*., 2012). The best way to give antioxidant nutrients to the human body is to eat generous servings of medicinal plants and vegetables rich in antioxidants such as polyphenols, tocopherols and tocotrienols. The protective effects of dietary phytochemicals against oxidative stress-related diseases are due to their contribution to the maintenance of redox homeostasis in cells. The aims of the present study were to screen the phytochemicals and investigate the free radical scavenging activity of some of Thai medicinal plants.

MATERIALS AND METHODS

Plant material preparation

Ten plant materials according to Table 1 were purchased from Charoensuk Osod, Nakorn Pathom Province, Thailand. The plants were washed and then dried in a hot air oven at 50 °C for 24 h. The dried materials were ground and passed through a sieve No. 45. The powders were kept in well-closed containers and protected from light at room temperature.

Table 1. Plant materials

Scientific names	Family	Part used
Cassia garrettiana (Craib.) Inwin & Basneby	Araliaceae	heartwood
Pinus merkusii Jungh et de Vriese	Leguminosae	heartwood
Dalbergia parviflora Roxb	Leguminosae	heartwood
Tarenna hoaensis Pitard	Rubiaceae	heartwood
Dracaena loureiri Gagnep	Dracaenaceae	heartwood
Thysostachy siamensis Gamble	Gramineae	root
Mimusops elengi L.	Sapotaceae	heartwood
Euphorbia antiquorum L.	Euphorbiaceae	heartwood
Piper ribesoides Wall	Piperaceae	stem
Oroxylum indicum (L.) kurz	Bignoniaceae	bark

Preparation of plant extracts

Each dried plant powders (50 g) were extracted with 200 ml of ethanol for 1 h with sonication method. The extracts were filtered through filter paper (Whatman® No. 1) and were then concentrated in vacuum. The obtained crude extract was kept in air-tight containers and protected from light.

Preliminary phytochemical screening

Ethanol extracts of ten plants were screened for the presence of flavonoids, phenolic compounds, saponins, terpenoids, alkaloids, anthraquinones, cardiac glycosides, tannins, reducing sugars and proteins by using standard procedures (Trease and Evans, 1989).

Test for phenolic compounds

Each ethanol extract (25 mg) was dissolved in ethanol (2 ml) and 2-4 drop of 5% ferric chloride solution was added. The presence of phenolic compounds was indicated by the appearance of a bluish black color.

Tested for flavonoids

Twenty five milligrams of ethanol extracts were dissolved in 2 ml of ethanol. A few drops of 10% NaOH solution were added into the extract solutions to give intense yellow color. One milliliter of concentrated sulphuric acid was added. The disappeared of yellow coloration after addition of concentrated sulphuric acid indicated the presence of flavonoids.

Test for terpenoids

Each ethanol extract (25 mg) was dissolved with 2 ml of chloroform. A few drop of concentrated sulphuric acid was carefully added to from layer. Formation of a reddish brown color of the interface indicated the presence of terpenoids.

Test for saponins

About 50 mg of each ethanolic extract was dissolved in water (5 ml) in test tube. The solution was shaken vigorously for 2 min and observed for a stable persistent froth indicated the presence of saponins.

Test for alkaloids

Twenty five milligrams of ethanol extract was boiled with 10% HCl solution (5 ml) for 15 min. The solution was filtered through filter paper, and 2 ml of dilute ammonia was added. The filtered was partitioned between chloroform. The chloroform layer was kept and extracted with 10 ml of acetic acid. Then, few drops of Dragendorff's reagent were added to the acetic acid extract. The formation of reddish brown precipitate was regarded as positive for alkaloids.

Test for cardiac glycosides

The ethanol extract (25 mg) was dissolved in 2 ml of water. Two milliliters of glacial acetic acid was added. One drop of 5% ferric chloride solution was added follow by addition of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar of cardenolides. An appearance of violate below the brown ring and greenish ring in acetic acid layer indicated the presence of cardiac glycosides.

Test for anthraquinones

The ethanol extract (25 mg) was boiled with 10 ml of 5% hydrochloric acid solution for 15 min and filtered through filter paper while hot. The filtered was partitioned between dichloromethane. The dichloromethane layer was collected into the test tube and 1 ml of 10% NaOH solution was added. A pink coloration indicates the presence of anthraquinones.

Test for tannins

One hundred milligrams of ethanol extract was boiled with 5 ml of water. The extract was filtered through filter paper. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue black coloration.

Test for reducing sugars

A small amount of each extract were weighed to 25 mg and dissolved in 5 ml of water in test tube. Two milliliters of Fehling's solution was added. The solution was boiled for 15 min and observed for reddish brown precipitation indicates the presence of reducing sugars.

Test for proteins

Fifty milligrams of extract was dissolved in 2 ml of distilled water and treated with Biuret reagent. Appearance of pink color indicated the presence of protein.

Determination of anti-oxidative activity of the extracts

DPPH free radical scavenging activity

The crude extract was dissolved in ethanol to yield a concentration of 500 mg/ml. The sample (1 ml) was pipetted to a test tube. The DPPH solution (0.2 mM) of 3 ml was added to the test tube and mixed for 30 s. The mixture was kept in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. Ethanol was used as a blank. BHA and BHT were used as standards. The experiment was done in triplicate. The % inhibition was calculated as follows.

$$\% \text{ inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where

$A_{control}$ = the absorbance of the blank

A_{sample} = the absorbance of the samples

Total reducing power

The antioxidant activity was determined by the FRAP method (ferric reducing antioxidant power). Ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex could be reduced by the compounds exhibiting the anti-oxidant activity. The product from the reaction, Fe^{2+}-TPTZ complex, was formed, and could be detected at 593 nm. The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 20 mM ferric chloride solution and 10 mM TPTZ (2,4,6-Tris(2-pyridyl)-1,3,5-triazine) solution in a ratio of 10:1:1. The mixture was warmed at 37 ºC for 4 min. Then, the extract solution samples of 20 µl prepared in ethanol at a concentration of 1 mg/ml were mixed with 150 µl of FRAP solution. The absorbance of the samples at 8 min was recorded at 593 nm. Gallic acid was used as a standard.

Determination of total phenolic contents

The total polyphenol content was determined using Folin-Ciocalteu reagent. The crude extract was dissolved in ethanol to produce a concentration of 1 g/ml. The extract solution of 1 ml was mixed with 1 ml of Na_2CO_3 (2%) solution at room temperature for 3 min. Folin-Ciocalteu reagent (50%) solution of 0.2 ml was added. The mixture was kept at

room temperature for 30 min, and the absorbance was determined at 750 nm. Gallic acid was used as a standard. Three independent experiments were assayed.

RESULTS AND DISCUSSION

Ten ethanol extract of Thai medicinal plants were screening for phytochemical constituents. All tested plant showed the presence of phenolic compounds, flavonoids and terpenoids. The ethanolic extract of *M. elengi* and *E. antiquorum* showed the negative result of tannins. Absence of reducing sugars was observed in ethanol extract of *P. merkusii*, *D. loureiri*, *E. antiquorum*, *D. parviflora* and *T. siamensis*. Saponins, alkaloids and cardiac glycosides were not found in any extracts and proteins were found in only ethanol extract of *C. garrettiana*. The positive result of anthraquinones was observed in *C. garrettiana* and *P. ribesoides* (Table 2).

Table 2. Phytochemical constituents of plant tested

Plants	RS	AN	TE	FL	PC	SA	TA	AL	CG	P
C. garrettiana	+	+	+	+	+	-	+	-	-	+
P. merkusii	-	-	+	+	+	-	-	-	-	-
D. parviflora	-	-	+	+	+	-	+	-	-	-
T. hoaensis	+	-	+	+	+	-	+	-	-	-
D. loureiri	-	-	+	+	+	-	+	-	-	-
T. siamensis	-	-	+	+	+	-	+	-	-	-
M. elengi	+	-	+	+	+	-	-	-	-	-
E. antiquorum	-	-	+	+	+	-	-	-	-	-
P. ribesoides	+	+	+	+	+	-	+	-	-	-
O. indicum	+	-	+	+	+	-	+	-	-	-

RS = reducing sugar; AN = anthraquinones; TE= terpenoids; FL = Flavonoids; PC = phenolic compounds; SA = saponins; TA = tannins; AL = alkaloids; CG = cardiac glycosides; P = proteins

+ = presence; - = absence

Antioxidant activity determined by DPPH method indicated that ethanol extract of the stem of *P. ribesoides* exhibited the most potent activity with the IC_{50} value of 5.61 ± 0.09 µg/ml, the ethanol extract of *C. garrettiana* showed the IC_{50} value of 7.31 ± 0.21 µg/ml. In addition, *P. ribesoides* and *C. garrettiana* showed more potent anti-oxidative activity than the standards, BHT and BHA.

The results from FRAP assay demonstrated that wood extract of *D. parviflora* (4,486 ± 101.27 mg GAE/100g sample) and stem extract of *P. ribesoides* (3,152.66 ± 57.74 mg GAE/100g sample) exhibited the highest anti-oxidative activity in term of reducing power. The data from both anti-oxidative activity methods were related (Table 3).

Table 3. Anti-oxidant activity of the ethanol extract of different plants tested by DPPH and FRAP assay

Plant	DPPH assay IC_{50} (µg/ml) (Mean ± SD)	FRAP assay (mg GAE/100g sample) (Mean ± SD)
C. garrettiana	7.31 ± 0.21	150.69 ± 17.35
P. merkusii	996.57 ± 3.42	393.76 ± 11.16
D. parviflora	38.02 ± 1.56	4,486.00 ± 101.27
T. hoaensis	67.11 ± 1.22	1,535.31± 33.23
D. loureiri	98.67 ± 1.52	1,235.11 ± 33.35
T. siamensis	47.59 ± 0.91	36.36 ± 6.98
M. elengi	1,199.64 ± 2.22	33.98 ± 0.98
E. antiquorum	53.33 ± 3.13	1,386.84 ± 24.61
P. ribesoides	5.61 ± 0.09	3,152.66 ± 57.74
O. indicum	25.01 ± 2.34	118.70 ± 5.75
Gallic acid	2.26 ± 0.11	-
BHA	41.93 ± 0.92	-
BHT	95.52 ± 1.91	-

Ethanolic extract of *C. garrettiana*, *D. parviflora*, *P. ribesoides* and *D. loureiri* yielded high total phenolic and flavonoid contents. *D. parviflora* showed the highest total phenolic content (5,130.74 ± 80.95 mg GAE/100g sample) and *D. loureiri* yielded the highest flavonoid contents (6,081.24 ± 67.71 mg GAE/100g sample) as shown in Table 4. The extracts exhibiting a good ability in anti-oxidative activity were found to contain higher phenolic and flavonoid contents. However, the anti-oxidative activity might also be resulted from other compounds in the extracts.

Table 4. Total phenolic and flavonoid contents in the ethanolic extracts of the plants

Plant	Total phenolic content (mg GAE/100g sample)	Flavonoid content (mg GAE/100g sample)
C. garrettiana	2,882.99 ± 135.10	1,965.29 ± 63.04
P. merkusii	399.35 ± 8.91	139.02 ± 63.84
D. parviflora	5,130.74 ± 80.95	1,990.72 ± 58.43
T. hoaensis	725.04 ± 6.08	371.12 ± 33.45
D. loureiri	3896.16 ± 13.41	6,081.24 ± 67.71
T. siamensis	458.43 ± 3.96	22.99 ± 4.43
M. elengi	326.99 ± 21.66	309.74 ± 13.84
E. antiquorum	1,838.32 ± 12.38	3,706.11 ± 52.78
P. ribesoides	1,258.05 ± 12.59	5,136.56 ± 117.08
O. indicum	264.57 ± 4.66	71.00 ± 3.24

CONCLUSION

The phytochemicals in ten Thai medicinal plant extracts were determined, and the antioxidant activity of these extracted was also investigated. The results of DPPH and FRAP assay revealed that extracts of *P. ribesoides* and *D. parviflora* exhibited the most potent antioxidant activity. The results of total phenolic and flavonoid contents were involved with the high potency of anti-oxidation of the plant extracts. The extracts showing high content of anti-oxidative compounds might be potential for use as the dietary supplements and could be used as the ingredients in cosmetic products.

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