Phytochemical Screening, Total Flavonoids, Tannins and Phenolic Compounds and Antioxidant Activities in *Oroxylum indicum* Fruit Extracts
การศึกษาสารพฤกษเคมี ปริมาณฟีนอลิกรวม แทนนินรวม และฟลาโวนอยด์รวม และฤทธิ์ต้านอนุมูลอิสระจากฝักต้นเพกา

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Abstract

Oroxylum indicum (*O. indicum*) is a plant used as traditional medicine in Thailand for treatments of gastric ulcer, skin diseases and tonsil pain for a long time. Thus, it is interesting to study phytochemical screening and the antioxidant activities in *O. indicum*. This research reported *O. indicum*, growing in Nakhon Ratchasima, Thailand, contained high amount of phenolic compounds, tannins and flavonoids. It also showed high antioxidant activities, EC_{50} 1.97 mg mL^{-1} which is closed to the well-known antioxidant, butylated hydroxytoluene (EC_{50} 1.69 mg mL^{-1}).

Keywords: *Oroxylum indicum*, phytochemical screening, antioxidant activity

บทคัดย่อ

เพกา (*Oroxylum indicum*) เป็นพืชสมุนไพรตามตำราแพทย์แผนไทยที่มีสรรพคุณใช้รักษาและป้องกันการเกิดโรคได้ เช่น สมานแผล ทำให้น้ำเหลืองปกติ ดับพิษโลหิต แก้ร้อนในกระหายน้ำ ช่วยระบายท้อง ช่วยบำรุงธาตุ แก้ท้องร่วง ประกอบกับเป็นพืชพื้นบ้านที่สามารถนำมารับประทานได้ในชีวิตประจำวันและมีการใช้ประโยชน์ด้านยารักษาโรคตามภูมิปัญญาชาวบ้านมาช้านาน จึงมีความน่าสนใจในการนำมาศึกษาสารออกฤทธิ์ที่อยู่ในส่วนต่างๆ งานวิจัยนี้เป็นการศึกษาสารพฤกษเคมี และฤทธิ์ต้านอนุมูลอิสระจากฝักเพกา จากการศึกษาสารสกัดเอทานอลจากฝักเพกาทั้งแบบสดและแบบแห้ง พบสารพฤกษเคมีหลายชนิด และมีสารประกอบฟีนอลิก แทนนิน และฟลาโวนอยด์ในปริมาณสูง และจากการศึกษาฤทธิ์ต้านอนุมูลอิสระด้วยวิธี DPPH radical scavenging พบว่าสารสกัดเอทานอลจากฝักเพกาสดมีฤทธิ์ต้านอนุมูลอิสระมากกว่าแบบแห้ง โดยมีค่า EC_{50} เท่ากับ 1.97 มิลลิกรัมต่อมิลลิลิตร ซึ่งมีฤทธิ์ต่ำกว่าสารต้านอนุมูลอิสระ butylated hydroxytoluene (EC_{50} 1.69 mg mL^{-1}) ที่ใช้โดยทั่วไปเพียงเล็กน้อย

คำสำคัญ: เพกา พฤกษเคมี ฤทธิ์ต้านอนุมูลอิสระ

Introduction

The antioxidants are well-known to be important for good health. The antioxidants reduce free radicals which cause cell damage in human body. These powerful substances mostly come from the fruits and vegetables including *Oroxylum indicum* (*O. indicum*). *O. indicum* is a medium-sized tree growing in China, South and Southeast Asia. This plant is used in Traditional Chinese Medicine, also in India and Thailand, for many medicinal properties e.g. anti-inflammatory, antipyretic, anticancer and anti-hypersensitivity (Uddin *et al*., 2003; Kumar *et al*., 2010; Yan *et al*., 2011; Dinda *et al*., 2015). In Thailand, *O. indicum* is a native plant used for treatments of gastric ulcer, skin diseases and tonsil pain. Therefore, it is interesting to study phytochemical screening and the antioxidant activities in *Oroxylum indicum* growing in Thailand.

Objectives

To study the phytochemical screening, total phenolic compounds, tannins, flavonoids and the antioxidant activities in *Oroxylum indicum.*

Scope of Research

The samples in this research are fresh and dried fruits of *O. indicum* growing in Tambon Sratakien, Amphur Sengsang, Nakhon Ratchasima, Thailand. The fruits were extracted in ethanol.

Methodology

1. Preparation of plant extract

O. indicum fruits were collected from Tambon Sratakien, Amphur Sengsang, Nakhon Ratchasima. The fruits were dried at 45 ^{o}C in hot air oven. Both fresh and dried fruits were extracted using 95% ethanol as solvent by maceration at room temperature for 7 days. The dried samples were obtained by filtration and evaporation of the extract, and ready for further studies.

2. Phytochemical screening

The phytochemical screening was determined by observing colors and precipitates from various reactions (Ayoola *et al*., 2008)

2.1 Detection of Flavonoids

0.2 g of an extract was added to 1.0 mL of 50% ethanol. The residue was removed by filtration and the filtrate was added with a small piece of magnesium and 5 drops of concentrated hydrochloric acid then warmed for 5 minutes. The solution would turn yellow as the presence of flavonoids.

2.2 Detection of Anthraquinones

0.2 g of an extract was added to 1 mL of 10% sulfuric acid. The mixture was mixed and warmed for 5 minutes then filtered out the residue. The filtrate was cooled to room temperature and mixed with 0.5 mL of 10% ammonia. The solution would turn pink as the presence of anthraquinones.

2.3 Detection of Coumarins

0.2 g of an extract was added to 1.0 mL of 50% ethanol. The residue was removed by filtration and 1mL of 6 M sodium hydroxide was added to the filtrate. The solution would turn yellow as the presence of coumarins.

2.4 Detection of Saponins

0.2 g of an extract was added to 5 mL distilled water and warmed for 5 minutes. The mixture was then shaken quickly and observed bubbles. The bubbles would be permanent as the presence of saponins.

2.5 Detection of Tannins

0.2 g of an extract was added to 5 mL distilled water and warmed for 5 minutes. The residue was removed by filtration and 5 drops of 1% iron (III) chloride was added to the filtrate. The solution would turn dark green or blue as the presence of tannins.

2.6 Detection of Phlobatannins

0.2 g of an extract was added to 5 mL distilled water and warmed for 5 minutes. The residue was removed by filtration and 5 drops of 10% hydrochloric acid was added to the filtrate then warmed for 5 minutes. The solution would turn dark green or blue as the presence of phlobatannins.

2.7 Detection of Terpenoids

0.2 g of an extract was added to 1 mL of chloroform and the mixture was filtered to remove the residue. Then, O.5 mL of concentrated sulfuric acid was slowly added to the filtrate (Do not shake). A brown circle would be observed between two layers as the presence of terpenoids.

2.8 Detection of Steroids

0.2 g of an extract was added to 1 mL of chloroform and the mixture was filtered to remove the residue. Then, 0.5 mL of glacial acetic acid was added to the filtrate, followed by 3 drops of concentrated sulfuric acid. The solution would turn green or blue as the presence of steroids.

2.9 Detection of Cardiac Glycosides

0.2 g of an extract was added to 1 mL of chloroform and the mixture was filtered to remove the residue. Then, 5 drops of 1% iron (III) chloride was added to the filtrate. The mixture was shaken and added with 0.5 mL of glacial acetic acid. Then, O.5 mL of

concentrated sulfuric acid was slowly added to the filtrate (Do not shake). A brown circle would be observed between two layers as the presence of cardiac glycosides.

3. Determination of Total Phenolic Content

Total phenolic content was determined by the Folin-Ciocalteu colorimetric method using Gallic acid as a standard reagent (Majhenic *et al*., 2007). Briefly, 0.2 mL of the standard Gallic acid solutions and extract solutions were added to 0.8 mL of Folin-Ciocalteu reagent 10% (v/v). The mixtures were incubated at room temperature for 5 minutes then mixed with 1 mL of 2.5% (w/v) sodium carbonate solution. After incubation at room temperature for 20 minutes, the absorbances of the solutions were measured at 760 nm using UV-Vis spectrophotometer. Quantification measurement was performed based on a standard calibration curve of 0, 20, 40, 60 and 80 mg/100 mL of Gallic acid in 80% ethanol**.** Total phenolic content was expressed as Gallic acid equivalent (GAE) in the 1 g of dry sample. Linear calibration curves were produced with R^2 = 0.9920.

4. Determination of Total Tannin Content

Total tannin content was determined by the Folin-Ciocalteu colorimetric method similar to the determination of total phenolic content using tannic acid as a standard reagent. 0.2 mL of the standard Gallic acid solutions and extract solutions were added to 0.8 mL of Folin-Ciocalteu reagent 10% (v/v). The mixtures were incubated at room temperature for 5 minutes then mixed with 1 mL of 2.5% (w/v) sodium carbonate solution. After incubation at room temperature for 20 minutes, the absorbances of the solutions were measured at 760 nm using UV-Vis spectrophotometer. Quantification measurement was performed based on a standard calibration curve of 0, 20, 40, 60 and 80 mg/100 mL of tannic acid in 80% ethanol**.** Linear calibration curves were produced with R^2 = 0.9829.

5. Determination of Total Flavonoid Content

Total flavonoid content was determined by the colorimetric method using Rutin solution as a standard reagent. Briefly, 5 mL of sample solution in 20% ethanol, 0.3 mL of 5% M sodium nitrite, and 0.3 mL of 10% aluminium chloride were added and mixed. The mixtures were incubated at room temperature for 5 minutes then mixed with 1 mL of 1 M sodium hydroxide. The absorbance of the mixture was measured at 510 nm using UV-Vis spectrophotometer Quantification measurement was performed based on a standard calibration curve of 0.0, 0.1, 0.2, 0.4, 0.8 and 1 mg/mL of Rutin solution. Linear calibration curves were produced with R^2 = 0.9987.

6. Determination of Antioxidant Activity by DPPH radical Scavenging Method

DPPH Free Radical Scavenging Method is modified from Braca, Sortino, Politi, Morelli and Mendez (Braca *et al*., 2002) using 1.8 mL of 0.05 mM DPPH (2,2-Diphenyl-1-picrylhydazyl) solution in methanol mixed to 0.2 mL of a sample extract or standard solution. The reaction mixture was incubated in the dark for 30 minute at room temperature and measured at 517 nm using an UV-Vis spectrophotometer. The percent DPPH Scavenging was calculated by an equation.

$$\% \text{ DPPH Scavenging} = \frac{\mathbf{A(control) - A(test\ sample)}}{\mathbf{A\ (control)}} \text{ X } 100)$$

EC_{50} is the required concentration of a sample to scavenge 50% of DPPH obtained from a graph between concentrations and % scavenging. EC_{50} of extracts were compared to standard antioxidants BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) (Williams *et al*., 1999).

Results

1. Yield of extracts

The ethanolic extract of *O. indicum* from fresh fruits were obtained as a dark green thick liquid while the extract from dried fruits were obtained as dark brown thick liquid. Yields of both extracts were shown in table 1.

Teble 1 Yield of *Oroxylum indicum* ethanolic crude extracts

Extracts	*Oroxylum indicum*		
	Mass of sample (g)	Mass of Extract (g)	Percent Yield (%)
Fresh friuts	500	44.01	8.8
Dried fruits	300	12.52	4.2

2. Phytochemical Screening

The ethanolic extracts of *O. indicum* from fresh fruits contained flavonoids, tannins and terpenoids while the extract from dried fruits contained flavonoids, coumarins, saponins, tannins, terpenoids and cardiac glycoside as shown in table 2.

Table 2 Phytochemical Screening of *O. indicum* ethanolic crude extract

Phytochemical Screening	*Oroxylum indicum*	
	Fresh fruits	Dried fruits
flavonoids	+	+
anthraquinones	-	-
coumarins	-	+
saponins	-	+
tannins	+	+
phlobatannins	-	-
terpenoids	+	+
steroids	-	-
cardiac glycoside.	-	+

2.1 Total Phenolic compounds

The total phenolic content in ethanolic extracts of *O. indicum* fruit was quantified and expressed in GAE (gallic acid equivalents). The fresh fruits and dried fruits contained total phenolic content 386.92 and 98.37 mg GAE g^{-1} crude extract respectively.

2.2 Total Tannin Content

The total tannin content in ethanolic extracts of *O. indicum* fruit was quantified and expressed in TAE (tannic acid equivalents). The fresh fruits and dried fruits contained total tannin content 519.03 and 134.28 mg TAE g^{-1} crude extract respectively.

2.3 Total Flavonoid Content

Total flavonoid content in ethanolic extracts of *O. indicum* fruit was quantified and expressed in RE (rutin equivalents). The fresh fruits and dried fruits contained total flavonoid content 2.84 and 2.92 mg RE g^{-1} crude extract respectively.

2.4 Antioxidant Activity by DPPH radical Scavenging Method

The ethanolic extracts of *O. indicum* fruits showed the antioxidant activity lower than standard BHA and BHT as shown in table 3. The dried fruits showed the higher antioxidant activity than the fruits.

Table 3 Antioxidant activity by DPPH radical scavenging method

Extracts	EC_{50} (mg/mL)
fresh fruits of *O. indicum*	1.97
dried fruits *O. indicum*	2.21
BHA	1.24
BHT	1.69

Conclusion

The ethanolic extracts of fresh *O. indicum* fruits contained total phenolic compounds, tannins, flavonoids content in a higher amount than dried fruits. Moreover, the fresh fruits showed higher antioxidant activity which is closed to the well-known antioxidant, BHT.

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