แทนนิน และองค์ประกอบทางพฤกษเคมีอื่นๆ ของใบปรงทะเล

Tannins and Other Phytochemical Constituents of *Acrostichum aureum* Linn. Leaves

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บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อหาปริมาณแทนนินและศึกษาองค์ประกอบทางพฤกษเคมีอื่นๆ ของใบปรงทะเลที่สกัดด้วยตัวทำละลายเฮกเซน เอทิลอะซิเตด 70% อะซิโตน เอทานอล และเมทานอล ผลการทดสอบพบว่าสารสกัดจากใบปรงทะเลพบสารสำคัญในกลุ่มแทนนิน แอลคาลอยด์ ฟลาโวนอยด์ คาร์ดิแอคไกลโคไซด์ และซาโปนิน โดยพบ คาร์ดิแอคไกลโคไซด์มากที่สุดในสารสกัดหยาบเมทานอล และพบซาโปนินมากที่สุดในสารสกัดหยาบเอทานอล ตรวจสอบปริมาณแทนนินด้วยวิธี Folin-Ciocalteu พบปริมาณแทนนินมากที่สุดในสารสกัดหยาบ 70% อะซิโตน มีค่าเท่ากับ 28.18 ± 0.025 มิลลิกรัมสมมูลของกรดแทนนิก/กรัมสารสกัด รองลงมาพบในสารสกัดหยาบเอทานอล เมทานอล เอทิลอะซิเตด และเฮกเซน มีค่าเท่ากับ 10.90 ± 0.010, 9.45 ± 0.006, 1.39 ± 0.010 และ 0.52 ± 0.020 มิลลิกรัมสมมูลของกรดแทนนิก/กรัมสารสกัด ตามลำดับ การวิจัยครั้งนี้เป็นข้อมูลเบื้องต้นที่ชี้ให้เห็นว่าสารสกัดหยาบของใบปรงทะเลมีสารมากมายสามารถแยกเพื่อหาสารสำคัญทางชีวภาพและมีศักยภาพที่จะพัฒนาเป็นสารต้านจุลชีพ ต้านอนุมูลอิสระ และต้านเชื้อราที่อยู่ในรูปของแทนนินได้ในอนาคต

คำสำคัญ: ปรงทะเล, พฤกษเคมี, แทนนิน

ABSTRACT

The purpose of this research was to determine the total tannins and phytochemical constituents of *Acrostichum aureum* Linn.leaves which was extracted in hexane, ethyl acetate, 70% acetone, ethanol and methanol. The result of phytochemical screening showed the presence of

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tannins, alkaloids, flavonoids, cardiac glycosides and saponins. Cardiac glycosides were highly concentrated in methanol extract and saponins were highly concentrated in ethanol extract. The analysis of total tannins content was performed by Folin-Ciocalteu method. The results suggested that the 70% acetone extract exhibited the highest total tannins content which was 28.18 ± 0.025 mg TAE/g extract, while the lower was detected in ethanol, methanol, ethyl acetate and hexane extract which was 10.90 ± 0.010, 9.45 ± 0.006, 1.39 ± 0.010 and 0.52 ± 0.020 mg TAE/g extract, respectively. This research is preliminary for demonstrating the potential of *A. aureum* leaves crude extract for biological active substances and develop as a significant source of natural antimicrobial, antioxidant and antifungal in the form of tannins in the future.

Key words: *Acrostichum aureum* Linn., phytochemical, tannins

INTRODUCTION

Medicinal plants are the richest bioresource of traditional systems for drugs and medicine, modern medicines, nutraceutical intermediates and chemical entities for synthetic drugs (Tiwari *et al*., 2011). The medicinal value of plants lies in some chemical active substances that produce a definite physiological action on the human body (Aiyelaagbe and Osamudiamen, 2009). A knowledge of the chemical constituents of plant is necessary for information about new sources of economic materials and further be valuable in discovering the actual value of folkloric remedies (Mojab *et al*., 2003). The active substances that extracted from these plants both support more safety than chemical synthetic substances and in addition to new alternative natural substances for food, medical and cosmetic entrepreneur. This probably led to the revival of interest in product from natural extracts. The plant extracts have many advantage active substances. The various phytochemical compounds detected were known to have beneficial importance in industrial and medicinal sciences.

Tannins are generally defined as naturally occurring polyphenolic compounds of high molecular weight to form complexes with the proteins. Tannin is reported to exhibit antiviral, antibacterial, anti-tumor activities and is also used as diuretic (Aiyelaagbe and Osamudiamen, 2009). Alkaloids are used as effective drugs and associated it to sedative properties and powerful effect on the nervous system (Renu, 2005). Flavonoids have been refered to act as antioxidant, hence it could offer protection against heart disease and cancer probably by enhancing the body defend against pathology induced free radicals generation (Ukoha *et al.*, 2011). Cardiac glycosides are used in the treatment of congestive heart failure and used to strengthen a weakened heart. Saponins are used in hyperglycaemia, antioxidant, anticancer and anti-inflammatory

etc. Anthraquinones are generally used as dyes and are also, known as antibacterial agents.

Acrostichum aureum Linn. is a mangrove in the family of *Pteridaceae*, which grow in marsh swamp environment or on mud flats of back water areas among coastal environment prevailing a tropical humid (Bonde and Kumaran, 2002). Traditional Thai folk user revealed that the stems of *A. aureum* used for abscess and neutralize treatment while the corms is treated for herpes simplex virus infection. Nevertheless, phytochemical study of this plant still insufficient information. The plant represents an enormous reservoir of biologically active compounds with various chemical structures and protective properties (Shakeri *et al*., 2012).

The objective of this study was to determine the total tannins by using Folin-Ciocalteau method and also phytochemical constituents of *A. aureum* leaves were screened in order to know the composition of various chemical active substances. The major chemical constituents of interest in these investigations were tannins, alkaloids, flavonoids, cardiac glycosides, saponins and anthraquinones. Moreover, these active substances will be useful many purposes, such as medical, food, cosmetics industrial for replace synthesis chemicals.

MATERIALS AND MATHODS

Plant Materials

The leaves of *A. aureum* were collected from mangrove forest at Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang province, Thailand in September 2013. The collected leaves were washed with distilled water to remove the dust and then were dried at 40 °C for 12 hours in hot air oven. The dried material was cut into small pieces and ground to powder.

Preparation of the Plant Extract

200 g of the powdered plant material was macerated in 500 ml of hexane at ambient temperature for one week. The extract was filtered and evaporated to dryness with rotary evaporator to give 6.08 g of crude extract. The other solvents were further studied. Powdered plant material was extracted with ethyl acetate, 70% acetone, ethanol and methanol to give 68.80, 105.48, 80.84 and 102.44 g of crude extracts, respectively. The crude extracts were obtained and further evaluated by phytochemical screening and analysis of total tannins.

Screening of Phytochemical Constituents

The phytochemical analysis such as tannins, alkaloids, flavonoids, cardiac glycosides, saponins and anthraquinones were carried out on hexane, ethyl acetate, 70% acetone, ethanol and methanol extract according to the common phytochemical methods (Benmehdi *et al*., 2012).

Screening for Tannins

The water extract of the crude dry

powder of each extract was treated with 2 drops of 2% ferric chloride solution. Blue dark color and precipitate indicated the presence of hydrolysable tannins.

The small amount of each extract was added with 5 mL of concentrated hydrochloric acid. The mixture was boiled for 15 minutes and filtered using a filter paper and collected in a beaker. Formation of red precipitate soluble in *iso*-amyl alcohol indicated the presence of condensed tannins.

Screening for Alkaloids

Alkaloids salts: small amount of extract was stirred with 15 mL of 10% hydrochloric acid on a steam bath for 30 minutes. The mixture was extracted for three times with diethyl ether. 1 mL of the aqueous layer was treated with two drops of Wagner's reagent. Formation of brownish precipitate indicated the presence of salts alkaloids.

Free Alkaloids: 10 mL of diethyl ether layer was evaporated to dryness. The residue was then dissolved in 1.5 mL of 2% hydrochloric acid and treated with two drops of Mayer's reagent. Turbidity and formation of creamy white precipitate indicated the presence of free alkaloids.

Screening for Flavonoids

A small piece of magnesium ribbon was added to small amount of extract, this was followed by the drop wise addition of concentrated hydrochloric acid. Colors varying from orange to red indicated the presence of flavones, red to crimson indicated the presence of flavonols, crimson to magenta indicated the presence of flavonones.

Screening for Cardiac Glycosides

Small amount of extract was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 mL of concentrated sulphuric acid. A brown ring of the interface as evidence for a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish-blue color ring may form just gradually throughout thin layer indicated the presence of cardiac glycosides

Screening for Saponins

Small amount of extract was introduced into a beaker containing 100 mL of distilled water, the mixture was boiled in a water bath and filtered. The filtrate was completed then to 100 mL with water. In ten test tubes were introduced the following volumes (1, 2, ..., 10 mL) of the mother solution. Then the final volume was readjusted to 10 mL with distilled water. All tubes were vigorously shaken for 15 seconds, formation of froth indicated the presence of saponins.

Screening for Anthraquinones

Small amount of extract was boiled with 10 mL of sulphuric acid and filtered while hot. The filtrate was shaken with 5 mL of chloroform. The chloroform layer was pipette into another test tube and 1 mL of dilute ammonia was added. Delicate rose pink color indicated the presence of anthraquinones.

Determination of Total Tannins

The amount of total tannins in five extracts were determined by Folin-Ciocalteu method (Tamilselvi *et al.*,2012). A calibration curve was plotted using tannic acid as standard. 1 mg of the sample extract was added with 7.5 mL of distilled water in test tubes, add 0.5 mL of Folin-Ciocalteu reagent, 1 mL of 35% sodium carbonate solution and dilute to 10 mL with distilled water. Vortex the tubes, kept at room temperature for 30 minutes and absorbance were measured at 725 nm. Blank was prepared with distilled water instead of sample. The results of tannins are expressed in terms of tannic acid mg TAE/g of extract.

Statistical Analysis

The determinations were conducted in triplicate and results were expressed as mean ± SD (standard deviation). Statistical analyses were done by one-way ANOVA followed by the Duncan's Multiple Range Test (DMRT) with $P < 0.05$ as a limit of significant.

RESULTS AND DISCUSSION

The percentage of extract content in 200 g of the powdered plant material in hexane, ethyl acetate, 70% acetone, ethanol and methanol are shown in Table 1.

Phytochemical analysis of *A. aureum* leaves extracts was determined by color reaction of the compounds with specific chemical reagent. The results of the interaction of extracts with the test reagents indicated the presence of alkaloids as alkaloid salts only in 70% acetone extract, flavonoids as flavones in 70% acetone and ethanol extract, while as flavonones in methanol extract, cardiac glycosides and saponins in all extracts. Besides, tannins were exhibited both condensed tannins and hydrolysable tannins which condensed tannins are known to be able to interact with biological systems through the induction of some physiological effects, such as antioxidant, anti-allergy, anti-hypertensive, as well as antimicrobial activities. Similarly, hydrolysable tannins are known for its ability to induce beneficial effects on human health through the expression of some biological activities, including antimutagenic, anticancer and antioxidant properties (Romani *et al*., 2006). The phytochemical constituents of hexane, ethyl

Table 1 Extract content of *A. aureum* in each solvent.

Solvent	% Extract content (w/w)
Hexane	3.04
Ethyl acetate	34.40
70% Acetone	52.74
Ethanol	40.42
Methanol	51.22

acetate, 70% acetone, ethanol and methanol extracts are shown in Table 2.

The total tannin contents of tannin-containing extracts are shown in Table 3. The standard curve built with tannic acid demonstrated that the type of standard used could influence seriously the results. The regression equation of the standard curve generated by Folin-Ciocalteu method was: $y = 0.005x + 0.037$; $R^2 = 0.997$ as shown in Fig 1.

For tannin contents, the values presented as the mean±SD of three measurements. It was found to be more in 70% acetone extract, while the lower was detected in ethanol, methanol, ethyl acetate and hexane extract respectively. In all extracts, the total tannins content were significantly different from each other as shown in Fig 2. The extractive capability of components from herb material is considerably depended on the type of solvent. The content of total tannins vary because of the different polarity and solubility of all of five extracts, as well as of the different polarity of the compounds found in the fractions and the solvent used for the extraction (ZahradNíkOVá *et al*., 2008). The extracts of some mangrove species indicated significant antioxidant activity and supposed the active compounds responsible.

Table 2 Phytochemical constituents screening of *A. aureum* leaves extracts

Phytochemical constituents	Results				
	Hexane extract	Ethyl acetate extract	70% Acetone extract	Ethanol extract	Methanol extract
Tannins					
- Condensed tannin	-	-	++	+	+
- Hydrolysable tannin	-	-	++	-	+
Alkaloids					
- Alkaloid salts	-	-	+	-	-
- Free alkaloids	-	-	-	-	-
Flavonoids					
- Flavones	-	-	+	+	-
- Flavonols	-	-	-	-	-
- Flavonones	-	-	-	-	+
Cardiac glycosides	++	+	++	+	+++
Saponins	+	+	++	+++	++
Anthraquinones	-	-	-	-	-

Note: – (Absence), + (Low in abundance), ++ (Moderate in abundance), +++ (High in abundance).

Table 3 Tannin contents of *A. aureum* leaves extracts.

Extract	A_{725}	Total tannins (mg TAE/g extract)
Hexane	0.089 ± 0.0020[e]	0.52 ± 0.020[e]
Ethyl acetate	0.176 ± 0.0010 [d]	1.39 ± 0.010[d]
70% Acetone	2.855 ± 0.0025 [a]	28.18 ± 0.025[a]
Ethanol	1.127 ± 0.0010 [b]	10.90 ± 0.010[b]
Methanol	0.982 ± 0.0006 [c]	9.45 ± 0.006[c]

* The values are given as mean ± standard deviation (n = 3).

** Means with different letter within a column are significantly different at $p < 0.05$ by the Duncan's Multiple Range Test (DMRT).

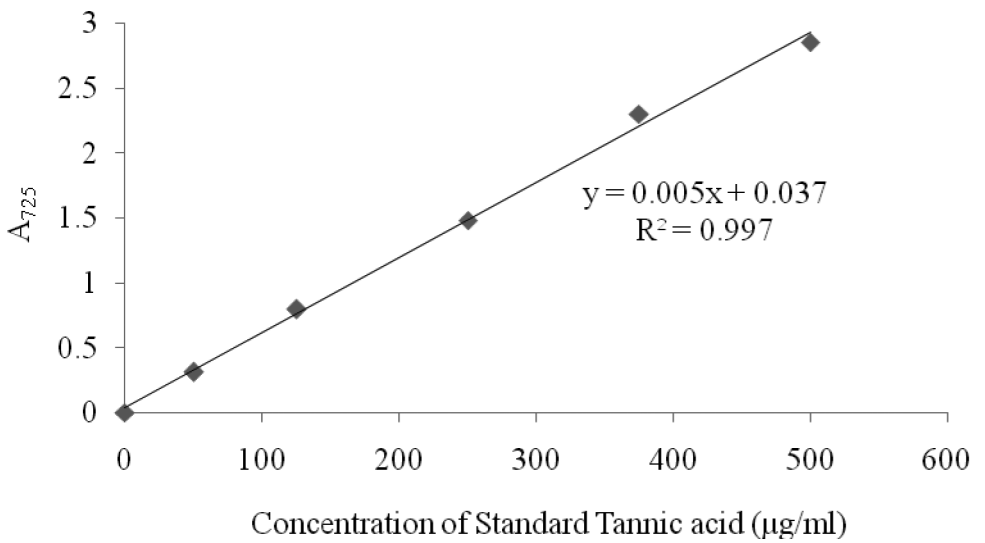

Figure 1 Calibration curve of standard tannic acid

The free radical scavenging activity increased with the increasing concentration of tannins (Zhang *et al*., 2010). Observation from the present study suggested that the tannins content is high in 70% acetone extracts as compared to ethanol, methanol, ethyl acetate and hexane extract, respectively corresponding to the sequence in antioxidant activity of *A. aureum* leaves which ethanol extract gave the highest antioxidant activity as compared to methanol, ethyl acetate and hexane extract respectively (Khongsai, 2013).

CONCLUSION

This research has shown that the qualitative analysis of the phytochemical screening in *A. aureum* leaves in hexane, ethyl acetate, 70% acetone, ethanol and methanol extract revealed the presence of tannins, alkaloids, flavonoids, cardiac glycosides and

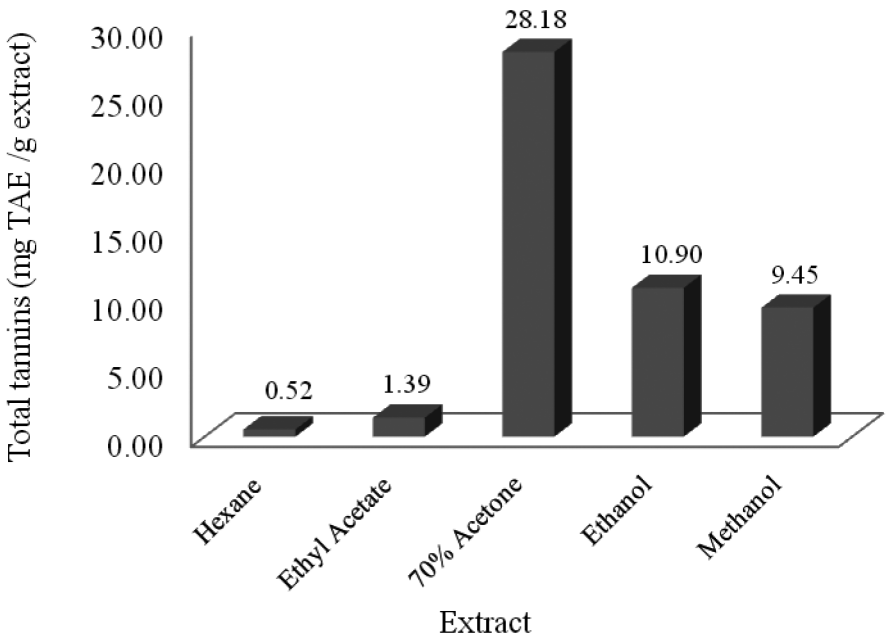

Figure 2 Total tannins (mg TAE/g extract) of *A. aureum* leaves extracts.

saponins. The determination of tannins contents has shown that the extraction using hexane, ethyl acetate, 70% acetone, ethanol and methanol have been successfully made. Results showed that the 70% acetone was the most efficient solvent among the five solvents used. The extraction variability reported between the solvents indicates that the selection of solvent is important and depends on the objective of the analyses to be performed on the extracts.

ACKNOWLEDGEMENTS

Funding for this research was provided by Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang, Thailand.

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