การตรวจสอบสารพฤกษเคมีเบื้องต้น การหาปริมาณฟีนอลิกและฟลาโวนอยด์ทั้งหมดและฤทธิ์ต้านอนุมูลอิสระจากต้นกุ่มน้ำ

PHYTOCHEMICAL SCREENING, DETERMINATION OF TOTAL PHENOLIC AND FLAVONOID CONTENTS AND ANTIOXIDANT ACTIVITIES FROM *CRATEVA MAGNA* (LOUR.) DC.

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บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อตรวจสอบหาสารพฤกษเคมีเบื้องต้น หาปริมาณสารฟีนอลิกทั้งหมด ฟลาโวนอยด์ทั้งหมดและทดสอบฤทธิ์ต้านอนุมูลอิสระในสารสกัดจากเอทานอลของดอก ใบอ่อน ใบแก่ กิ่ง และเปลือกจากต้นกุ่มน้ำ *Crateva magna* (Lour.) DC. ทดสอบฤทธิ์ต้านอนุมูลอิสระด้วยวิธี DPPH, ABTS และ FRAP หาปริมาณสารฟีนอลิกทั้งหมดและฟลาโวนอยด์ทั้งหมดใช้วิธี Folin-Ciocalteu และ Aluminium chloride colorimetric ตามลำดับ ผลการตรวจสอบหาสารพฤกษเคมีเบื้องต้นพบสารฟีนอลิก ฟลาโวนอยด์ แทนนินและน้ำมันหอมระเหยอยู่ในสารสกัดจากส่วนต่าง ๆ ของพืช ตัวอย่างสารสกัดจากใบอ่อนให้ปริมาณฟีนอลิกทั้งหมดและฟลาโวนอยด์ทั้งหมดมากที่สุดเมื่อเปรียบเทียบกับสารสกัดอื่นมีค่าเท่ากับ 5.73 ± 0.28 mg GAE/g DW และ 56.62 ± 0.23 mg RUE/g DW ตามลำดับโดยวิธี DPPH และ ABTS มีค่า IC_{50} เท่ากับ 0.30 ± 0.02 และ 0.92 ± 0.04 mg/ml ตามลำดับ และมีค่า FRAP เท่ากับ 13.06 ± 0.08 $FeSO_4$/g DW สรุปได้ว่าสารสกัดจากใบอ่อนของพืชชนิดนี้มีปริมาณสารฟีนอลิกและฟลาโวนอยด์ที่สูงและมีคุณสมบัติต้านอนุมูลอิสระที่ดีมากเมื่อเทียบกับสารสกัดอื่น ๆ และยังพบว่าปริมาณสารฟีนอลิกและฟลาโวนอยด์มีความสัมพันธ์ต่อฤทธิ์ต้านอนุมูลอิสระ ดังนั้นสารสกัดจากกุ่มน้ำจึงเป็นแหล่งสารต้านอนุมูลอิสระที่ดีและสามารถนำไปพัฒนาในด้านเภสัชภัณฑ์ได้ต่อไป

คำสำคัญ: กุ่มน้ำ พฤกษเคมี ฤทธิ์ต้านอนุมูลอิสระ

Abstract

This research aims to perform phytochemical screening, determine total phenolic content, flavonoid content and antioxidant properties of ethanol extracts of flowers, young leaves, mature leaves, twigs and bark of *Crateva magna* (Lour.) DC. The antioxidant activity was studied using DPPH, ABTS and FRAP assays. The total phenolic content (TPC) and total flavonoid content (TFC) of the extracts were determined using Folin-Ciocalteuand and Aluminium chloride colorimetric methods, respectively. The

preliminary phytochemical test results indicated the presence of phenolics, flavonoids, tannins and volatile oils in the extracts from different parts of the plant samples. The young leaves extract possessed the highest value of TPC and TFC with 5.73 ± 0.28 mg GAE/g DW and 56.62 ± 0.23 mg RUE/g DW, respectively. The young leaves extract also showed the highest antioxidant activity as evidenced by DPPH and ABTS assays at IC_{50} values of 0.30 ± 0.02 mg/ml and 0.92 ± 0.04 mg/ml, respectively and FRAP value of 13.06 ± 0.08 $FeSO_4$/g DW. In conclusion, the young leaves extract of this plant showed high phenolic and flavonoid contents with strong antioxidant properties. It was also found that phenolic and flavonoid contents related to antioxidant activity. Therefore, the extracts from *C. magna* are good sources of antioxidants and could be developed for pharmaceutical applications.

Keywords: *Crateva magna* (Lour.) DC., phytochemical, antioxidant activity

Introduction

Currently, antioxidants are gaining attention from people around the world. Many years ago, there were several studies that showed antioxidants can help prevent many kinds of diseases for example cancer, cardiovascular, neurological disorders, diabetes and immunological disorders (Yadav et al., 2016). Generally, antioxidants are widely distributed in plants such as fruits, vegetables, tea, spices and herbs which occur in all parts of plant (Mandal et al. 2009; Kumar, 2014; Embuscado, 2015). Several studies have demonstrated the importance of the compounds from plants with antioxidant activity. Phenolic and flavonoid compounds are large groups of phytochemicals that have powerful antioxidant activity (Saxena et al., 2012). Phenolic and flavonoid compounds, secondary metabolites of plants with primary antioxidant properties, present potential health benefits. Besides, many studies have suggested that phenolic acids and flavonoids exhibit other biological activities like antiallergenic, antiviral, anti-inflammatory, and vasodilating actions. So, these compounds are an important group of plant based biologically active compounds (Pietta et al., 2000; Shahidi et al., 2012; Banjarnahor et al., 2014).

Crateva magna (Lour.) DC. belongs to the family Capparaceae, a medicinal plant commonly known as "Kum nam" in Thailand. The parts of this plant are widely used in traditional medicine to treat various diseases: the leaf (fever), bark (hemorrhoids), heart wood (kidney stones and to drain pus), root (thirst relief) and the flowers and young leaves are used as food (Chuakul, 2010; Neamsuvan et al., 2015). The ethanol and aqueous extracts from the bark showed significant antiurolithiatic activity in *vivo* (Mekap et al., 2011; Ramu et al., 2017). The leaves, stem and bark exhibited significant antioxidant and anti-glucosidase activities (Loganayaki & Manian,

2012). It contains various phytochemicals, including phenolics, flavonoids, terpenoids, steroids, glycosides, sugars, alkaloids, quinones, coumarins, saponins and lignins (Behera et al., 2016; Abirami et al., 2017; Radha & Doraiswamy, 2017). Therefore, this research is aimed to screen phytochemicals, determine the content of phenolics and flavonoids, and to compare the antioxidant capacities obtained by DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt and FRAP (ferric reducing antioxidant potential) assay in the flowers, young leaves, mature leaves, twigs and barks of *C. magna*. The findings from this research may add to the overall value for this plant.

Materials and methods

Plant materials

The parts of the plant used in this study were flowers, young leaves (deep brown leaves), mature leaves (deep green leaves), twigs and bark of *C. magna*. These samples were collected in February, 2018 from Pichit province, Thailand and were dried at room temperature. A voucher specimen was deposited at the Faculty of Science and Technology, Pibulsongkram Rajabhat University, Phitsanulok, herbarium code number PSRU0308.

Chemicals and reagents

Folin-Ciocalteu's phenol reagent was purchased from Loba chemie Pvt. Ltd (Mumbai, India). 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis (3-ethylbenzothiazoline -6-sulfonic acid) diammonium salt (ABTS) and 2,4,6-tripyridyl-s-triazine (TPTZ) reagents were purchased from Sigma-Aldrich (Gillingham, UK). Ascorbic acid, ferric chloride, aluminium chloride and potassium acetate were purchased from Ajax Finechem (New South Wales, Australia). Ethanol (AR. Grade), acetic acid (AR. Grade), hydrochloric acid (AR. Grade), sodium hydroxide and sulfuric acid were purchased from Lab scan (Bangkok, Thailand). Sodium acetate was purchased from Riedel-de Haen (Seelze, Germany). Gallic acid was purchased from Fluka chemika (Steinhein, Germany). Sodium carbonate was purchased from Fisher Scientific (Leicestershire, UK).

Extract preparation

Fifty grams of dried powder from each sample were macerated in ethanol at room temperature for 7 days (2×200 ml). After filtration, the combined extracts were concentrated with rotary evaporator at 45 °C to obtain flowers extract (4.88 g, 9.75%), young leaves extract (4.17 g, 8.34%), mature leaves extract (1.83 g, 3.66%), twigs extract (3.53 g, 7.05%) and bark extract (2.39 g, 4.77%).

Preliminary phytochemical screening

All extracts were identified to various classes of phytochemicals by the method from Radha & Vijayakumari (2013). Six chemical compounds of phenolics, flavonoids, tanins, anthocyanins, quinones and volatile oils were tested. Phenolics compound was tested with ferric chloride ($FeCl_3$) by using 2 drops of 5% ferric chloride with 1 ml of the extract. The positive result was shown as the formation of deep blue or black.The presence of flavonoids was confirmed by adding 1 ml of the extract to 2 drops of aqueous sodium hydroxide (NaOH) and 2 drops of hydrochloric acid (HCl). The orange color formation was indicated as a positive test for the presence of flavonoids. For tannin, 2 ml of water was added to 1 ml of extract, boiled and filtered. Then, 2 drops of 5% ferric chloride were added to the filtrate. A deep green, blue or brown indicated the presence of tannins. Extracts were tested for anthocyanins with 2 drops of 2M sodium hydroxide. Appearance of blue green color indicated the presence of anthrocyanins. For quinone, 1 ml of extract was tested with 2 drops of concentrated HCl. The formation of a yellow precipitate showed the presence of the quinones. For volatile oil, 2 ml of extract solution was shaken with 0.1 ml of dilute sodium hydroxide and a small quantity of dilute HCl. Formation of white precipitate indicated the presence of volatile oils.

Determination of total phenolic content (TPC)

The TPC of different parts of *C. magna* extracts was determined using the Folin-Ciocalteu colorimetric method (Wanyo et al., 2014). Briefly, the reaction mixture was composed of 0.2 ml of extract, 1.8 ml of the Folin-Ciocalteu reagent. After a period of 5 minutes, 1.5 ml of saturated sodium carbonate solution was added. This mixture was shaken and allowed to stand for 90 minutes. The absorbance was measured at 725 nm. TPC was calculated as mg gallic acid equivalents per gram of dry weight (mg of GAE/g DW) by using a gallic acid calibration curve.

Determination of total flavonoid content (TFC)

The TFC of samples was determined using the method from Brighents et al. (2005). Briefly, 3 ml of 2% aluminium chloride ($AlCl_3$) in ethanol was mixed with the 5 ml of the sample. Absorbance readings at 415 nm were taken after 10 minutes at room temperature. The results were expressed as mg rutin equivalents per gram dry weight (mg of RUE/g DW).

Determination of DPPH radical scavenging ability

Antioxidant activity was determined by the DPPH method which was the modification of the method from Kaur et al. (2017). Briefly, 1 ml of the extract (1.000 to 0.156 mg/ml) was added to 2 ml of 0.2 mM DPPH solution. All solutions obtained were then incubated for 30 minutes at room temperature. Absorbance was measured at 517

nm. The radical scavenging capacity using the free DPPH radical was evaluated by measuring the decrease of absorbance at 517 nm. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample

Determination of ABTS radical scavenging ability

The free radical-scavenging activity of sample was determined by ABTS assay described by Pang et al. (2018). The stock solutions were 7 mM ABTS and 2.45 mM potassium persulfate. The ABTS solution was then prepared by mixing the two stock solutions in equal proportion and allowing them to react for 16-18 hours. This solution was stored in a dark place at room temperature. Before use, the ABTS solution was diluted with ethanol (1:20 v/v). Crude extracts and fractions (0.3 ml) were allowed to react with 3 ml of ABTS solution and measured at 754 nm. The radical scavenging capacity using the free ABTS radical was evaluated by measuring the decrease of absorbance at 754 nm. The inhibition percentage of ABTS radical was calculated using the following formula:

$$\text{ABTS scavenging activity (\%)} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

Ferric reducing antioxidant power assay (FRAP)

The antioxidant capacity was determined following the procedure described by Pramai & Jiamyangyen (2016). The FRAP reagent was freshly prepared by adding 10 mM of 2,4,6-Tripyridyl-s-triazine (dissolved in 40 mM HCl), 20 mM of $FeCl_3$ in water and 300 mM of acetate buffer (pH 3.6) in the ratio of 1:1:10 (v/v). The FRAP reagent, 1 ml, was mixed with 20 µl of test sample (1.000 to 0.156 mg/mL) and 1 ml of H_2O (37 °C) or standard solution. The absorbance of the Iron (II) sulfate solution was measured at 593 nm after 4 minutes incubation at 37 °C. The antioxidant capacity based on the ability to reduce ferric ions from the sample was calculated from the linear calibration curve and expressed as mg $FeSO_4$ equivalents per gram of dry weight (mg $FeSO_4$/gDW).

Statistical Analysis

The data was analyzed by one way analysis of variance (ANOVA) and the differences between samples were determined by Duncan's Multiple Range test (SPSS version 20 for Windows). Values were expressed as mean ± S.D., n = 3. Mean value within each group with different letters (a-e) indicated significant differences with $p < 0.05$.

Results

Results for screening of phytochemicals are shown in Table 1. Six classes of phytochemical screening tests including phenols, flavonoids, tannins, anthocyanins, quinones and volatile oils were found in ethanol extract of flowers, young leaves, mature leaves, twigs and bark from *C. magna*. Exceptionally, volatile oils were not found in the bark extract.

Table 1 Phytochemical screening of the different parts from *C. magna*.

Phytochemicals	Sample				
	Flowers	Young leaves	Mature leaves	Twigs	Bark
Phenols	+	+	+	+	+
Flavonoids	+	+	+	+	+
Tannins	+	+	+	+	+
Anthocyanins	-	-	-	-	-
Quinones	-	-	-	-	-
Volatile oils	+	+	+	+	-

Remark + indicates presence; - indicates absence

Total phenolic and flavonoid contents (TPC, TFC)

The results of TPC from five crude extracts are shown in Table 2. Standard gallic acid calibration curved was found to be suitable (R^2 = 0.996) The TPC in the investigated crude extracts ranged from 0.81 ± 0.09 to 5.73 ± 0.28 mg GAE/g DW. The concentrations of flavonoids reacted with aluminum chloride and compared with the standard rutin equivalents gram of dry weight was calculated from the calibration curve (R^2 = 0.999). The TFC in the investigated crude extracts ranged from 11.61 ± 0.04 to 56.62 ± 0.23 mg RUE/g DW.

Table 2 Total phenolic and flavonoid contents in the ethanol extracts of different parts of *C. magna.*

Crude extracts	Total phenolic content (mg GAE/g DW)	Total flavonoid content (mg RUE/g DW)
Flowers	4.77^{c} ± 0.30	38.49^{d} ± 0.14
Young leaves	5.73^{d} ± 0.28	56.62^{e} ± 0.23
Mature Leaves	1.70^{b} ± 0.09	25.53^{b} ± 0.16
Twigs	0.87^{a} ± 0.11	11.61^{a} ± 0.04
Bark	0.81^{a} ± 0.09	31.52^{c} ± 0.18

Remark Different letters indicate significances between groups ($p < 0.05$). Values represent means ± S.D. (n =3)

Antioxidant activities by DPPH, ABTS and FRAP assay

The antioxidant capacity of different parts of *C. magna* was determined using DPPH, ASTS and FRAP assays. The DPPH and ABTS methods are reported in terms of the half maximal inhibitory concentration (IC_{50}) value corresponding to the sample concentration that reduced the initial DPPH and ABTS absorbance of 50%. The IC_{50} values of DPPH and ABTS were expressed in ranges from 0.30 ± 0.02 to 3.86 ± 0.14 and 0.92 ± 0.04 to 3.02 ± 0.16 mg/ml, respectively.The result of FRAP from all crude extracts and compared with the standard solutions of the Iron (II) sulfate, calculated from the calibration curve (R^2 = 0.999). The FRAP of the investigated crude extracts ranged from 5.14 ± 0.37 to 13.06 ± 0.08 mg $FeSO_4$/g DW. The results are presented in the Table 3.

Table 3 DPPH, ABTS and FRAP assay of the ethanol extracts of different parts of *C. magna.*

Crude extracts	DPPH assay IC_{50} (mg/ml)	ABTS assay IC_{50} (mg/ml)	FRAP assay (mg $FeSO_4$/g DW)
Flowers	0.55^{b} ± 0.06	1.68^{b} ± 0.03	$12.25^{c,d}$ ± 0.36
Young leaves	0.30^{a} ± 0.02	0.92^{a} ± 0.04	13.06^{d} ± 0.08
Mature Leaves	0.73^{d} ± 0.01	1.61^{b} ± 0.03	5.14^{a} ± 0.37
Twigs	0.68^{c} ± 0.04	2.24^{c} ± 0.02	11.56^{c} ± 0.21
Bark	3.86^{e} ± 0.14	3.02^{d} ± 0.16	6.01^{b} ± 1.06

Remark Different letters indicate significances between groups ($p < 0.05$). Values represent means ± S.D. (n =3)

Discussion

The preliminary phytochemical screening of ethanol extracts obtained from different parts of *C. magna* showed that they included phenolics, flavonoids, tannins and volatile oils. These compounds are known as a source for pharmaceutical application. Especially, phenolics and flavonoids have been reported to have the presence of biological activities such as anti-inflammatory activity, antimicrobial activity, antibacterial activity etc. (Tapas et al., 2008). The present study has revealed that the ethanol extract obtained from the young leaves exhibited the highest antioxidant activity and it also contained the highest amount of phenolics and flavonoids. Therefore, it could be inferred that phenolics and flavonoids are responsible for antioxidant activity which is in agreement with several reports that have shown close relationship between these compounds and antioxidant activity in fruits, plants and vegetables (Adebiyi et al., 2017). This study employed three different antioxidant methods for accurate confirmation of their antioxidant properties. Thus, this plant was found to be a good source of active compounds which could be developed for use in

pharmaceutical industry. Further research is needed in terms of isolation, purification and characterization of the active compounds.

Conclusions

The results suggested that *C. magna* was found to be rich in the phytochemicals. All extracts showed antioxidant activities and in correlation with the TPC and TFC. Therefore, they could be the principle groups of bioactive compounds. It was also found that the use of DPPH assay coupled with other methods such as ABTS and FRAP could be able to accurately determine antioxidant properties. Therefore, the extracts from *C. magna* are considered as a good source of antioxidants and could be developed for use in pharmaceuticals.

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