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Correlation between total flavonoid content and total phenolic content on antioxidant activity of ethanol extracts from three cultivars of papaya leaves

Prakit Chaithada*[1], Praweena Whenngean[1], Radchada Fungfueng[1], Saowanee Maungchanburee[2]

[1]General Science, Faculty of Education, Nakhon Si Thammarat Rajabhat University, Nakhon Si Thammarat 80280, Thailand

[2]Biomedical Sciences, Faculty of Medicine, Prince of Songkla University, Songkhla 90110, Thailand

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ABSTRACT

The aim of this study was to elucidate the correlation between total flavonoid content and total phenolic content on antioxidants activity of ethanol extracts from three cultivars of papaya leaves: 'Holland,' 'Khak Dam' and 'Red Lady.' All crude extracts were investigated to find antioxidant capacity in DPPH radical scavenging. The result indicated that the ethanol extract of 'Red Lady' papaya leaves exhibited the highest level of DPPH radical scavenging activity with the IC_{50} of 0.18 mg/mL, followed by the 'Khak Dam' and 'Holland' papaya leaves having an IC_{50} value of 0.24 and 0.44 mg/mL, respectively. The ethanol extract from 'Red Lady' papaya leaves showed the highest level of total flavonoid content (TFC) of 276.72 $\pm$ 1.04 μgQE/g DW and total phenolic content (TPC) of 169.85 $\pm$ 6.54 mgGAE/g DW. All three cultivars showed a distinctive correlation between IC_{50} and the content of both total flavonoid and total phenolic with a negative relationship of Pearson's correlation of -0.922 and -0.940, respectively.

*Corresponding Author

Name: Prakit Chaithada
Phone: +6691-8477042
Email: prakit_cha@nstru.ac.th

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INTRODUCTION

Free radicals are molecules or atoms that have a single electron inside the molecule. It is an unstable and reactive substance that affects human health. Free radicals react with biomolecules in the body, causing changes in genetic material and various processes in the body resulting in abnormalities. The reaction of free radicals in the body of a human is harmful to the brain, immune system and cardiovascular system. This causes the induction of diseases such as diabetes, premature aging, cancer, high blood pressure, heart disease and degeneration of the nervous system (Fan *et al.*, 2017), which are considered the top diseases in Thailand and around the world.

Antioxidants should be prescribed in an easy and understandable way for all relevant communities. In simple terms, antioxidants are inhibitors or slow down unwanted oxidation reactions. Current analysis methods for measuring antioxidants and antiviral activity are organized in various perspectives (Apak, 2019). Nutritional value can be assessed from the antioxidant activity and vitamin C content contained in extracts from vegetables, fruits and various natural products (Chaiwon *et al.*, 2013). Antioxidant components of plants play an important role, which may be effective as a therapeutic agent (Poojary *et al.*, 2016). Plant extracts and essential oils are gaining increasing attention because natural antioxidants have little impact on human health and the environment, suitable for

application in the pharmaceutical and food industry (Samet *et al.*, 2019). Medicinal plants have many active substances such as flavonoids, tannins and polyphenols. Phenolic compounds are divided into three main groups according to the chemical structure: hydroxycitric acid, phenolic acids, and flavonoids (Kim *et al.*, 2006). They are a kind of phytochemicals, which have a wide variety of nutritional and health properties, such as hypoglycemic activity, hypolipidemic activity and antibacterial activity (Kemperman *et al.*, 2013; Shahidi and Ambigaipalan, 2015). The antioxidation, total phenolic and total flavonoid content of various plant extract depending on the extraction methods, extracting solvents, botanical origin and seasons (Rebaya *et al.*, 2015; Chaithada *et al.*, 2018; Nascimento *et al.*, 2018). Green leafy plants are rich in antioxidants, helping to inhibit a variety of tumors and reduce the risk factors of heart disease (Pandiyan *et al.*, 2019). In the current investigation, we determined the total phenolic content (TPC) and total flavonoid content (TFC) of ethanol extracts from three cultivars of papaya leaves. We also investigated the correlation between both contents on antioxidant activity.

MATERIALS AND METHODS

Chemicals and Instrument

Ethanol and methanol were purchased from Merck Ltd. The Standard compound of flavonoids content (quercetin), of phenolic content (gallic acid), 2,2-diphenyl-1-picrylhydrazyl (DPPH), aluminum chloride ($AlCl_3$), potassium acetate (CH_3COOK), Folin-Ciocalteu's reagent and sodium carbonate (Na_2CO_3) were acquired from Merck Ltd. All of the chemicals and solvents were of analytical reagent grade (AR grade). The extraction solvents were evaporated by rotary evaporator (Buchi Rotavapor® R-300) from Buchi (Thailand) Ltd. and the colorimetric methods were measured using UV-Vis spectrophotometer (Spectroquant® Prove 300) from Merck Ltd.

Plant material

Three cultivars of papaya leaves ('Holland,' 'Khak Dam' and 'Red Lady') were collected from Khao Phanom District, Krabi province, Phrasaeng district, Surat Thani province and Pa Payom district, Phatthalung province, respectively.

Preparation of plant extracts

The samples of all three cultivars of papaya leaves were cut into small pieces and grinded thoroughly, then dried in a hot air oven at 60°C for 17 hours. The three cultivars of papaya leaves powder weighed 20 grams were extracted with soxhlet extractor using 200 ml of ethanol for 4 hours, then evaporate the extracted solvent using a rotary evaporator.

DPPH radical scavenging assay

The inhibition of free radicals was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. A Slightly modified method was used following the method of (Wasman *et al.*, 2011) and (Palafox-Carlos *et al.*, 2012). A DPPH methanolic solution (0.2 mM) was mixed with various concentrations of the extract sample (0.1-0.5 mg/mL). After 30 minutes of leaving in the dark place at room temperature, the optical density was measured at 517 nm using a UV-Vis spectrophotometer.

$$\%\ inhibition\ =\ \left[\frac{Abs_{control}-Abs_{sample}}{Abs_{control}}\right]\ \times\ 100$$

Determination of total phenolic content

The total phenolic content (TPC) was determined by using Folin-Ciocalteu reagent (FCR) with a slight modification of (Liu *et al.*, 2013). Gallic acid was used as a standard for this assay. Concisely, 0.5 mL of ethanolic extract (1 mg/mL) was added with 2.5 mL of 10% Folin-Ciocalteu reagent (FCR). After 5 minutes, 2 mL of 7.5% Na_2CO_3 was mixed with the solution. The mixture was allowed to stand for 1 hr in the dark at room temperature. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer. The total phenolic content was estimated from the standard curves of gallic acid. The results were presented as mg of gallic acid equivalent (GAE) per g of dry weight using the following formula:

$$Total\ phenolic\ content\ (TPC)\ =\ \frac{GAE\times V\times D}{W}$$

Whereas: V is the sample volume used for assay in mL, D is dilution factor and W is the weight of the extract in grams.

Determination of total flavonoid content

The total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method with some modification of (Bag *et al.*, 2015). Quercetin was used as a standard for this assay. Shortly, 0.5 mL of ethanolic extract (20 mg/mL) was mixed with 1.5 mL of methanol, 0.1 mL of 1 M potassium acetate, 0.1 mL of 10% aluminum chloride and diluted with 2.8 mL of distilled water. All solutions were filtered through Whatman filter paper. The optical density was determined at 415 nm using a UV-Vis spectrophotometer. The total flavonoid content was estimated from the standard curves of quercetin, and the results were presented as μg of quercetin equivalents (QE) per g of dry weight using the following formula,

$$Total\ flavonoid\ content\ (TFC)\ =\ \frac{QE\times V\times D}{W}$$

Whereas: V is the sample volume used for assay in mL, D is dilution factor and W is the weight of the extract in grams.

Statistical analysis

All assays were performed in triplicate, and data were presented as the mean ± standard deviation (SD). Analysis of correlation was conducted using SPSS Statistics version 21.

RESULTS AND DISCUSSION

The extraction yields of the ethanol obtained per 20 grams of dry leaves of papaya are shown in Table 1.

Table 1: The extraction yields of ethanol from three cultivars of papaya leaves

Papaya sample	Weight of sample (g)	Ethanol extracts	
		Weight (g)	% yield
Holland	20.00	5.23	26.15
Khak Dam	20.00	7.91	39.55
Red Lady	20.00	5.71	28.55

The yields of ethanol extract of Khak Dam cultivars were the highest at 7.91%, followed by Red Lady and Holland cultivars at 5.71 and 5.23%, respectively.

The free radical inhibition of each extract was analyzed. DPPH method was determined by preparing the sample at concentrations of 0.10, 0.20, 0.30, 0.40 0.50 and 1.00 mg/mL and determined at 517 nm wavelength by spectrophotometer compared with vitamin C standard solutions. Antioxidant reacted with DPPH causing the solution to change the color from purple to yellow. The comparison of the antioxidant capacity of each cultivar is determined by the relationship between %DPPH radical scavenging activity and concentration of papaya leaves extract, as shown in Figure 1.

The percentage of DPPH radical scavenging is linearly in the 0.2-0.5 mg/mL concentration range. These concentration ranges are plotted in linear graphs to find the half-maximal inhibitory concentration (IC_{50}), giving results, as shown in Figure 2.

It was found that the ethanol extract from 'Red Lady' papaya leaves exhibited strong antioxidant capacity with an IC_{50} value of 0.18 mg/mL, followed by the 'Khak Dam' papaya leaves having an IC_{50} value of 0.24 mg/mL. While the 'Holland' papaya leaves exhibited moderate levels of antioxidant activity with an IC_{50} value of 0.44 mg/mL.

The total phenolic content and total flavonoid content tended to similar, with both being the most

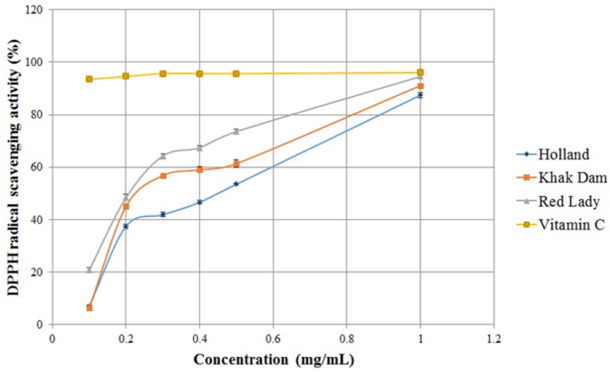

Figure 1: The average DPPH radical scavenging activity of papaya leaves extracts to compare with vitamin C

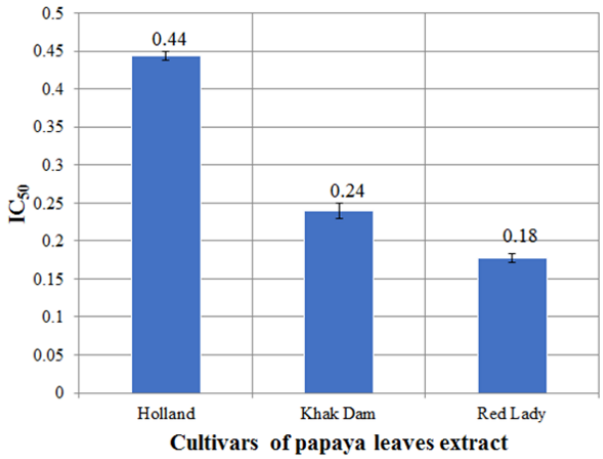

Figure 2: The half-maximal inhibitory concentration (mg/mL) of ethanol extracts from three cultivars of papaya leaves

common in ethanol extract form 'Red Lady' papaya leaves at 169.85 ± 6.54 mgGAE/g DW and 276.72 ± 1.04 μgQE/g DW, respectively. Ethanol extracts from 'Khak Dam' papaya leaves showed the total phenolic content and total flavonoids content were 84.99 ± 4.61 mgGAE/g DW and 155.45 ± 0.88 μgQE/g DW, respectively, while 'Holland' papaya extracts had the lowest values of 13.38 ± 1.91 mgGAE/g DW and 69.88 ± 0.77 μgQE/g DW, respectively as shown in Table 2.

Table 2: Total phenolic content and total flavonoid content of papaya leaves extracts

Papaya crude extracts	Total phenolic content, mgGAE/g DW	Total flavonoid content, μgQE/g DW
Holland	13.38c ± 1.91	69.88c ± 0.77
Khak Dam	84.99b ± 4.61	155.45b ± 0.88
Red Lady	169.85a ± 6.54	276.72a ± 1.04

The analysis of the correlation between total pheno-

lic content and the antioxidant capacity from Pearson's relationship showed that the total phenolic content had a negative relationship with the IC_{50} (r = -0.940), as shown in Figure 3(a). The papaya leaves extract with high total phenolic content had a low IC_{50} value, indicating high antioxidant capacity. The correlation between total flavonoid content and antioxidant capacity is expressed in the same way. The papaya leaves extract with high total flavonoid content had a low IC_{50} value with a Pearson's correlation value of -0.922 (Figure 3b).

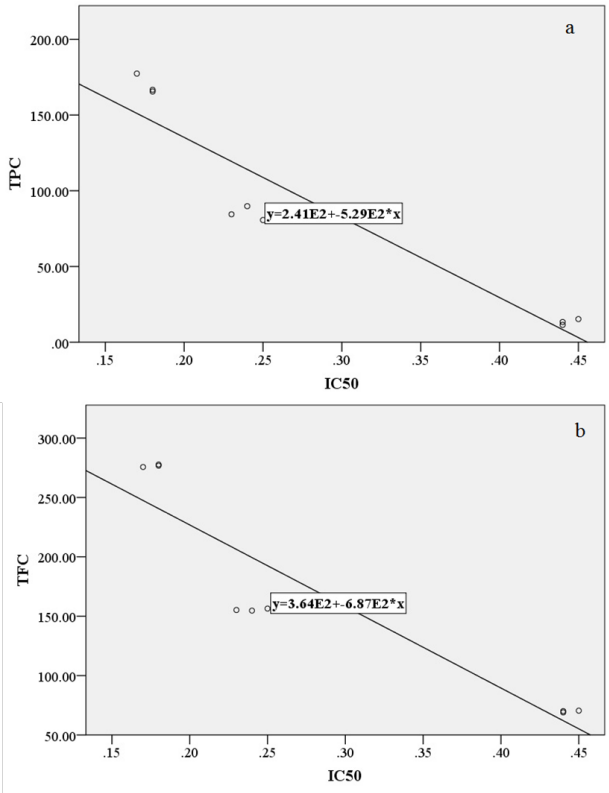

Figure 3: Correlation between the half-maximal inhibitory concentration (mg/mL) of ethanol extracts from three cultivars of papaya leaves (a-total phenolic content; b-total flavonoid content)

The studies on antioxidant activity of 3 papaya leaves: 'Holland,' 'Khak Dam' and 'Red Lady' have shown that extracts of ethanol from leaves 'Red Lady' exhibits strong antioxidant capacity with the IC_{50} value of 0.18 mg/mL. The 'Red Lady' papaya leaves extract shows the highest of total phenolic and flavonoid content at 169.85 $\pm$ 6.54 mgGAE/g DW and 276.72 $\pm$ 1.04 μgQE/g DW, respectively. Similar to the study of the antioxidant activity of ethanol extract from the *Ocimum gratissimum* Linn. leaves contained high total flavonoid and total phenolic content and showed potent antioxidant activity in three *in vitro* system: DPPH, nitric oxide scavenging assay and reducing power assay (Upadhyay and Bhagwat, 2014). Analysis of the relationship between antioxidant activity and TPC shows that all phenolic content has a negative relationship with IC_{50} (positive correlation with the percentage of free radical scavenging). From papaya leaves with high total phenolic content with low IC_{50} values, indicating high antioxidant capacity. The relationship between antioxidant activity and TFC is expressed in the same way. Papaya leaves extract with a high total flavonoid content has a low IC_{50} value. Mangiferin is a natural polyphenol of the structure. C-glycosylxanthone and its pharmacological effects, which can be found in many plants.

The relationship between mangiferin, TPC, TFC and the antioxidant activity is at a high level from 0.77 to 0.97 ($p<0.05$). This important positive relationship indicates that the antioxidant activity of *Phaleria macrocarpa* varies with the proportional change of concentrations of mangiferin, TPC and TFC (Lim *et al.*, 2019). DPPH activity has a strong relationship with phenolic compounds. The study of phenolic compounds contained in the seed coat, cotyledon and embryo of 9 soybean strains (*Glycine max* L.) showed that DPPH activity had a strong correlation with phenolic compounds (Kim *et al.*, 2006). As well as the study of the free radical inhibition of the leaves and flower crude extracts of *Halimium halimifolium*, which was determined by DPPH, ABTS and FRAP, showed a linear relationship with the content of polyphenols and flavonoid content (Rebaya *et al.*, 2015).

CONCLUSION

The study reveals that the papaya leaves in ethanol extract have strong antioxidant activity, while the 'Red Lady' papaya leaves show the best antioxidant activity, followed by the 'Khak Dam' and 'Holland' cultivars, respectively. The total flavonoid and total phenolic content were negatively correlated with the IC_{50} with Pearson's correlation of -0.922 and -0.940, respectively.

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