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DPPH RADICAL SCAVENGING ACTIVITY, TOTAL PHENOLICS AND FLAVONOIDS OF WATER SOLUBLE EXTRACTS DERIVED FROM LEAVES AND FRUIT OF *Ficus carica* L. AND *Ficus parietalis* Bl.

AKTIVITAS ANTIRADIKAL DPPH SERTA PENENTUAN KANDUNGAN FENOLIK DAN FLAVONOID TOTAL SARI LARUT AIR DAUN DAN BUAH *Ficus carica* L. DAN *Ficus parietalis* Bl.

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ABSTRACT

Ficus carica L and Ficus parietalis Bl. (Moraceae) are closely related plants which also known in Indonesia as Figs L. Considering the wide therapeutic value of Figs, this research was aimed to evaluate the DPPH-radical scavenging activity of both plants as well as their total phenolic and flavonoids. Extracts were produced by using boiled water and diluted to gain the desired concentration. Analyses were performed by using UV-vis spectrophotometer. Radical scavenging activity testing was done by using radical of DPPH (2,2-diphenyl-1-pycrylhydrazyl) to determine the IC_{50}s. The determination of total phenolic was conducted by using Folin-Ciocalteau method and calculated as Gallic Acid Equivalence (GAE). The total flavonoid was measured by using $AlCl_3$-reagents, and calculated as Rutin Equivalence (RE). Afterwards, the radical scavenging activity was correlated to the total phenolic and flavonoids contents. The results showed that the water soluble extract of F. carica fruit had the best IC_{50} value of 33.38 mg/mL, followed successively by the F. parietalis fruit (35.69 mg/mL), F. parietalis leaves (44.01 mg/mL) and the F. carica leaves (76.38 mg/mL). The highest content of total phenolic was shown by the leaves of F. parietalis (1.46% w/w GAE) and the lowest was in the fruit or F. carica (0.36% w/w GAE). The highest flavonoid content was detected in the leaves of F. carica (1.42% w/w RE) and the lowest was in the F. parietalis fruit (0.20% w/w RE). Correlation analyses of the IC_{50} values vs. the total phenolic and the flavonoids contents resulted in a positive slope having R^2 values as 0.5362 and 0.9895, respectively. As a conclusion, the total flavonoid content influenced the DPPH radical scavenging activity by 98.95%, while the total phenolic content influence was only 53.62%.
Keywords: *Ficus carica* L., *Ficus parietalis* Bl., DPPH-radical scavenging activity, total phenolic, total flavonoid

ABSTRAK

Tumbuhan Ficus carica L. dan F. parietalis Bl. (Moraceae) adalah tumbuhan yang di Indonesia kerap kali disebut sebagai Tin atau Ara. Mengingat penggunaannya yang sangat luas bagi pengobatan, penelitian ini bertujuan untuk mengetahui aktivitas antioksidan serta kandungan fenolik dan flavonoid total dari sari larut air daun dan buah F. carica dan F. parietalis Ekstrak diperoleh dengan cara menyari sejumlah sampel menggunakan air mendidih sehingga diperoleh konsentrasi yang diinginkan. Pengujian aktivitas penangkapan radikal dilakukan dengan menggunakan DPPH (2,2-difenil-1-pikrilhidrazil) dan ditentukan nilai $IC_{50.}$ Penentuan kandungan fenolik total dilakukan menggunakan pereaksi Folin Ciocalteau dan dinyatakan sebagai ekuivalen asam galat. Flavonoid total ditetapkan dengan menggunakan reagen $AlCl_3$ dan dinyatakan sebagai ekivalen rutin (ER). Kandungan fenolik dan flavonoid yang didapatkan selanjutnya dikorelasikan dengan aktivitas antiradikal. Hasil penelitian menunjukkan bahwa nilai IC_{50} terbaik terdapat pada buah F. carica L. (33,38 mg/mL), kemudian buah F. parietalis B. (35,69 mg/mL), daun F. parietalis Bl. (44,01 mg/mL), dan daun F. carica L. (76,38 mg/mL). Kandungan fenolik tertinggi terdapat pada daun F. parietalis Bl. (1,46% b/b EAG) dan terendah pada buah F. carica L. (0,36% b/b EAG). Kandungan flavonoid tertinggi terdapat pada daun F. carica L. (1,42% b/b ER) dan terendah pada

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buah F. parietalis Bl. (0,20% b/b ER). Analisis korelasi antara nilai IC_{50} dengan kandungan fenolik dan flavonoid total menunjukkan korelasi positif dengan nilai R^2 sebesar 0,5362 dan 0,9895 dengan kemiringan positif. Dengan demikian dapat disimpulkan bahwa kandungan flavonoid total mempengaruhi aktivitas penangkal radikal bebas sebesar 98,95%, sedangkan pengaruh kandungan fenolik total hanya sebesar 53,62%.

Kata kunci: *Ficus carica L., Ficus parietalis Bl., aktivitas penangkapan radikal DPPH, total fenolik, total flavonoid*

INTRODUCTION

There are at least 800 species of Ficus known worldwide. Part of the plants, the bark, root, leaves, fruit and latex are often used to overcome various diseases. *Ficus* species are known to be rich of poly phenolics and flavonoids which are correlated to the antioxidant capacities (Sirisha *et al*., 2010). Regardless of various reports which have been made for these plants, reports of those planted in Indonesia are still rare. *F. carica* L. and *F. parietalis* Bl. are two of genus *Ficus* which are found in Indonesia, both associated to Figs.

F. carica L. have been widely known as a rich source of phenolics with high antioxidant capacity, which are potential to overcome many diseases. (Solomon *et al*., 2006). Anti-HSV, haemostatic, hypoglicemic, and hypo-lipidemic (Chawla *et al*., 2012), anticancer (Rubnov *et al*., 2001), antipyretic (Patil *et al*., 2010) are some of tremendous reports on the plants bioactivities. However, on the contrary, the reports on *F. parietalis* Bl. pharmacological activities are still very rare.

Local people usually consume the fruits directly, or after being processed or dried, while the dried leaves are boiled or consumed as tea. Therefore, in order to provide scientific proof of the traditional usage of both plants, this research focused on the water soluble extracts of *F. carica* L. and *F. parietalis* Bl.

METHODOLOGY

Materials

Leaves and fruits of *F. carica* and *F. parietalis* were kindly provided by Drs. Djaetun H. S. (Klaten, Indonesia). DPPH radical (2,2-diphenyl-1-pycrilhydrazyl) (Calbiochem®), aluminum pre coated silica gel RP 18 (Merck, Germany), Rutin (Sigma Aldrich, Germany, 90% of purity), Gallic Acid (Wako Pure Chemicals Industry, Japan, 99% purity), Folin-Ciocalteu reagent (Merck, Germany), $AlCl_3$ (Merck, Germany), natrium acetate, natrium carbonate, ethyl alcohol p.a, methanol p.a, formic acid, $FeCl_3$, distilled water.

Equipments

Oven, electric balance (Metttler teledo, Switzerland), UV-Vis spectrophotometer (Hitachi U-2900, Japan), delivery pipette (Gilson pipetmen, USA), blue tip, yellow tip.

Methods

Sample preparation

Taxonomy determination was performed in the Pharmacognosy Laboratory, Faculty of Pharmacy, UGM under registration No. BF/295/Ident/Det/VI/2015.

Samples were washed, sliced, air-dried, followed by oven drying at 60˚C for 24 h. Dried leaves were grinded while the fruits were sliced into smaller size. Boiled water was used to extract the samples. Samples in amount of 0.5 g each was extracted by using 5mL of boiling water.

Phytochemical screening

Water extracts from leaves were spotted on to the aluminum precoated silica gel RP 18 TLC plate. Elution in methanol - water - formic acid (1:1:0.1 v/v) as the mobile phase was performed on the samples. Spot detections were done by using UV 254 nm, UV 366 nm lamps, and spraying reagents: $AlCl_3$, $FeCl_3$, anisaldehyde H_2SO_4, 2,4-DNPH, Dragendorf, KOH-ethanolic, and DPPH.

Water extracts from fruit were treated as aforementioned method. However, due to a poor spots separation on the TLC, the extracts were partitioned by using ethyl acetate. Each of 1 mL of sample was fractionated with 2.5 mL ethyl acetate. The solvent was evaporated and the resulted fraction was spotted onto the silica gel RP 18 plate and eluted by ethanol – water – formic acid (1:1:0.1 v/v) as the mobile phase. Detection was done as described previously for the leaves samples.

In order to detect the presence of saponin, as much as 0.5 g sample was each put into a tube and 5 mL of boiled distilled water was added and shaken vigorously for 30 sec. Stable foam for around 30 min is classified as saponin containing sample.

Tannin content was detected by extracting 0.5 g of sample by 5 mL boiled distilled water; afterwards, 500 µL of the extract was added with 1 mL gelatin. Tannin containing sample will cause sedimentation of the gelatin.

Radical Scavenging Activity Assay (modified from Kikuzaki *et al.*, 2002)

DPPH solution 0.4 nM was obtained by putting 15.8 mg of DPPH into a 100 mL flask of which ethanol p.a was added to reach the 100 mL mark and vortexed. Sample in amount of 0.25 mL was put into a 5 mL flask of which 1 mL of DPPH 0.4 mM and ethanol were added to reach the mark, and vortexed. After 30 min of incubation, absorbance was measured at 515.5 nm. Blank used was ethanol p.a. Control used was 1 mL of DPPH 0.4 mM.

$$\%\ \text{radical scavenging activity} = \left(\frac{\text{absorbance control} - \text{absorbance sample}}{\text{absorbance control}}\right) \times 100\ \%$$

Total Phenolic Content Determination (modified from Singelton, 1999)

Sample in amount of 0.25 g was extracted by 10 mL boiled water, filtered. As much as 0.5 mL of the sample solution was added with 2.5 mL Folin-Ciocalteu reagent, left for 4 min and then added with 2 mL 7.5%. $NaCO_3$. After 120 min incubation, the absorbance was measured at 760 nm. Blank used was the reagents alone without sample addition. Total phenolic contents were stated as gallic acid equivalence. Standard curve was made in the range of 25-125 µg/mL of gallic acid (R > 0.99).

Total Flavonoid Content Determination (modified from Chang *et al.*, 2002)

Sample in amount of 0.5 g sample was extracted with 10 mL boiled water and filtered. As much as 0.5 mL of the solution was added 1.5 mL ethanol and 0.1 mL 10% $AlCl_3$, 0.1 mL 1 M sodium acetate and 2.8 mL distilled water. After 30 min of incubation, the absorbance was measured at 416.5 nm. Total flavonoids were expressed as Rutin Equivalence. Standard curve was made in the range of 50-250 µg/mL rutin (R > 0.99).

Data Analyses

Shapiro-Wilk and ANOVA or *Kruskal-Wallis* was used to evaluate the significance of radical scavenging activity, total phenolics and total flavonoid contents. Significance level of 95% was used (α = 0.05). Correlation analysis was used to analyze the correlation between the radical scavenging activity vs total phenolics and total flavonoid contents.

RESULT AND DISCUSSION

Leaves and fruits of *F. carica* L. and *F. parietalis* Bl. (Moraceae) exhibit different plant morphology which can be easily distinguished (Figures 1 and 2). Qualitative phytochemical screening of leaves and fruits of *F. carica* and *F. parietalis* showed different chemical profiles of both plants samples (Table I-II; Figures 3-4).

Figure 1. Leaves and fruits of *Ficus carica* L. (a) and *Ficus parietalis* Bl (b).

Figure 2. *Ficus carica* L (left) and *Ficus parietalis* Bl. (right)

Phytochemical screening has shown that both leaves and fruits contain substances positive to scavenge DPPH radicals. The water soluble fraction of *F.carica* leaves contains more compounds which scavenge the DPPH and consisted of flavonoids and phenolics. On the other hand, only flavonoid was detected in the *F. parietalis* water soluble extract of the leaves of which showed a weak DPPH radical scavenging activity. The ethyl acetate fraction of the water soluble extract of the fruits, both of *F.carica* and *F. parietalis*, did not show radical scavenging activity, but the water fractions showed stronger activity.

Table I. TLC profiles of *F. carica* and *F. parietalis* water soluble extracts derived from the leaves

Sample	hRf	Before spraying		After spraying		
		UV 254	UV 366	$AlCl_3$	$FeCl_3$	DPPH
F.parietalis leaves	36	+	Dark violet	Dark yellow	-	+
	45	+	Dark violet	Yellow	-	-
F.carica leaves	6	+	Yellow	Yellow	Yellow	+
	11	+	Blue	Blue	Blue	+
	30	+	Dark violet	Dark yellow	Dark violet	+
	35	+	Blue	Blue	Blue	+
	43	-	-	Dark violet	Dark violet	-
	46	+	Yellow	Dark Yellow	-	+
	53	-	-	Yellow	-	-
Rutin	31	+	Brown	Dark yellow	*	++
Gallic acid	80	+	Dark violet	*	Dark violet	++

Notes: +: Weak intensity; ++: Strong intensity ; *: not performed

Table II. TLC profiles of *F. carica* and *F. parietalis* ethyl acetate fraction of water soluble extracts derived from the fruits

Sample	HRf	Before spraying		After spraying		
		UV 254	UV 366	$AlCl_3$	$FeCl_3$	DPPH
F.parietalis fruit	3	+	Blue	Blue	Blue	-
	38	-	Yellow	Yellow	Yellow	-
	55	-	Yellow	Yellow	Yellow	-
	64	+	-	-	-	-
	69	+	-	-	-	-
	75	+	-	-	-	-
A. carica fruit	3	+	Blue	Blue	Blue	-
	18	-	Pale yellow	-	-	-
	30	-	Pale yellow	-	-	-
	38	-	Yellow	Yellow	Yellow	-
	55	-	Yellow	Yellow	Yellow	-
	64	+	-	-	-	-
	69	+	-	-	-	-
	75	+	-	-	-	-
Rutin	71	+	Brown	Dark yellow	*	++
Gallic acid	81	+	Dark violet	*	Dark violet	++

Notes: + : Weak intensity; ++ : Strong intensity ; * : not performed

However, poor TLC spots separation of the water extract has limited the chemical compound group's identification. Negative results were observed following spraying of the TLC plate of the ethyl acetate fraction with anisealdehyde H_2SO_4, 2,4-DNPH, Dragendorf, and KOH-ethanolic, as well as following saponin and tannin tube tests.

Nakilcioglu and Hisil (2013) reported that the *F. carica* is rich in calcium, natrium, ascorbic acid, vitamin A, fiber, fatty acid, and a lot of phenolics. The leaves contain bergapten, 4',5'-dihydropsoralen, rutin, 24-metilensikloartanol, umbelliferone, marmesin, stigmasterol, β-sitosterol, ficusogenin, lupeol, fiber, and carotenes. The fruits contain cyanidine-3-*O*-glucoside, cyanidine-3-*O*-rhamnoglucoside, saturated fat, sterol, sugar, protein, vitamin A, vitamin C, calsium and iron (Chawla *et al*., 2012). *F. parietalis* Bl. has been reported to contain polyphenol, flavonoid glycoside, alkaloid, prosalen, tannin, steroid, and vitamin. The fact that many of the above reported groups of compounds were not detected is probably caused by the compounds being relatively less polar, so that they cannot be well-extracted by boiled water, and or the contents are very small to be detected. Boiled water is expected to be able to extract glycosides, tannin, saponin, water soluble vitamins and sugars from the samples.

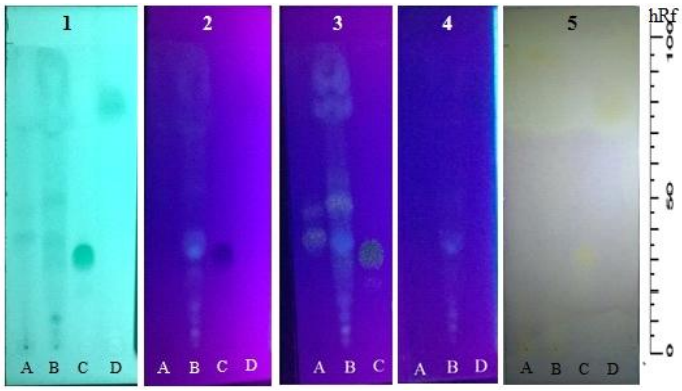

Figure 3. TLC Profiles of *F. carica* and *F. parietalis* water soluble extracts of the leaves
Notes: 1. UV 254; 2. UV 366; 3. UV 366 after sprayed with $AlCl_3$; 4. UV 366 after sprayed with $FeCl_3$; 5. Visible after sprayed with DPPH; *A.* Extract of *F. parietalis* Bl. Leaves; *B.* Extract of *F. carica* L. leaves; *C.* Rutin; *D.* Gallic acid.

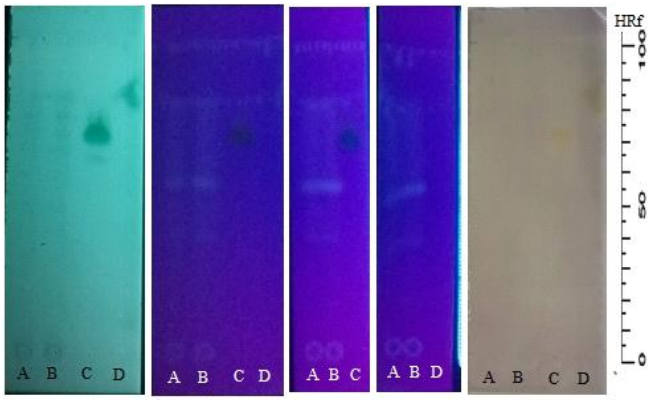

Figure 4. TLC Profiles of *Ficus carica* L. and *Ficus parietalis* Bl. ethyl acetate fraction of the fruit's water soluble extracts

Table III. IC_{50} values, total phenolics and flavonoid contents of water soluble extracts derived from leaves and fruits of *Ficus carica* L. and *Ficus parietalis* Bl.

Sample	IC_{50} (mg/mL) ± SD	Antioxidant capacity*	Total phenolics content (% w/w GAE) ± SD	Total flavonoid content (% w/w RE) ± SD
F.parietalis leaves	44.01± 0.211	Weak	1.46 ± 0.006	0.61 ± 0.010
F.carica leaves	76.38 ± 0.246	Weak	1.43 ± 0.006	1.42 ± 0.006
F.parietalis fruit	35.69 ± 0.112	Weak	0.51 ± 0.001	0.20 ± 0.001
F. carica fruit	33.38 ± 0.245	Weak	0.36 ± 0.001	0.25 ± 0.003
Rutin	0.07 ± 0.000	Strong		

Notes: * According to Ariyanto, 2006

Radical scavenging activity assays showed that all the samples exhibited weak activities in comparison to Rutin as the positive control (Table III). However, fruits samples showed higher radical scavenging activity in comparison to the leaves of which very polar substances are responsible to the activity (based on the phytochemical screening). In order to evaluate the active compounds in the water soluble extracts, total phenolics and flavonoids were determined separately. The phenolics in general, those including the flavonoids, have been correlated to antioxidant activity of plants. However, the Folin-Ciocalteau reagents reacts with other reducing compounds in the samples as well e.g. amino acids, sugar.

Gallic acid was used as a reference standard in the total phenolics measurement. Resulted formula was Y = 0.0062 X – 0.0103, R = 0.9992 of which Y was the absorbance while X was the gallic acid concentration. Rutin was used as a standard for flavonoid content determination. The resulted formula was Y = 0.0025 X + 0.028, R = 0.9992, of which the Y was the absorbance and the X was the rutin concentration.

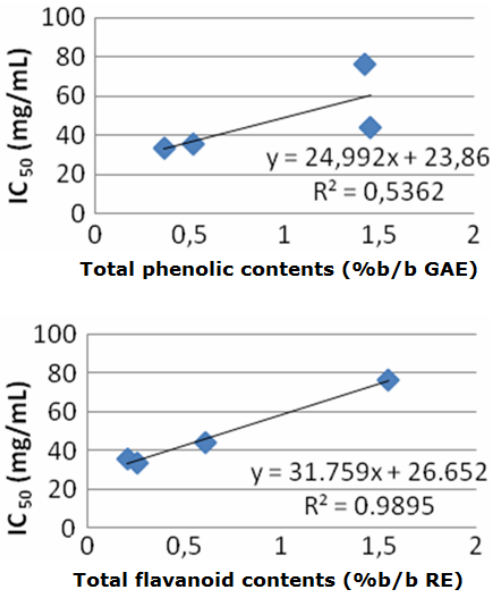

Figure 5. Correlation diagram of total phenolics (first chart) and total flavonoid (second chart) contents vs IC_{50} value of water soluble extracts derived from leaves and fruits of *Ficus carica* L. and *Ficus parietalis* Bl.

Correlation analyses of the radical scavenging activity vs total phenolics and total flavonoids contents have both resulted positive correlation, of which the total flavonoids caused much stronger correlation in comparison to the total phenolics contents (Fig 5). This phenomenon can be explained by different type of phenolics and flavonoids present in the samples which may provide different activities of each compound alone and as addition effects of several compounds. This fact was supported by different chromatogram profiles observed on the TLC. Sugars and ascorbic acid, might be the responsible chemical contents which might contribute to the reduction potential of the samples which are soluble in water, especially of the fruit extracts. Solomon *et al*. (2006) reported that the poly-phenols including the anthocyanin in *F. carica* fruit has a positive correlation to the antioxidant activity. However, other reports stated that the Fig leaves contain phenolics which has been reported elsewhere as potential antioxidant, i.e., hydroxycinnamic acids (3- and 5-*O*-caffeoylquinic acids and ferulic acid), a flavonoid glycoside (quercetin 3-*O*-rutinoside) and the furanocoumarins (psoralen and bergapten) (Oliveira *et al*., 2009, 2012) and chlorogenic acid (Teixeira *et al*., 2009). Besides, Vallejo and collaborators (2012) have reported that the concentration of total phenolics in skin fruit is higher than in flesh. The antioxidant of the fruit can protect the plasma lipoprotein from oxidation and significantly increase the plasma antioxidant capacity for 4 h after consumption (Vinson *et al*., 2005). A strong correlation between the phenolics and the antioxidant activity of the Fig leaves has been reported by several authors (Çaliskan and Polat, 2011; Oliveira *et al*., 2009; Konyalioglu *et al*., 2005; Veberic *et al*., 2008; Mahmoudi *et al*., 2016). Despite being a closely related plant, reports on *F. parietalis* is much less than those of *F. carica*. Research on the anti-oxidative substances contain in this plant and other close related Figs is worth exploring.

CONCLUSION

The results have shown that the water soluble extract of *F. carica* fruit exhibited the highest radical scavenging activity in comparison to other samples The IC_{50} value of the *F. carica* fruit was 33.38 mg/mL, while the fruit of *F. parietalis* Bl. was 35.69 mg/mL; the leaves of *F. parietalis* Bl. was 44.01 mg/mL, and the leaves of *F. carica* was 76.38 mg/mL. The activity was 53.62% correlated to the total phenolics contents while the correlation to the total flavonoids contents to the DPPH scavenging activity was higher as shown by 98.95% correlation.

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