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The Influence of Growing Environment on the Total Phenol Content and Antioxidant Activity of *Ficus hispida* Leaves and Fruits

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Abstract: Antioxidant activities in the leaves and fruits of *Ficus hispida* from different growing environment of Malaysia (Tasik Chini, Tasik Bera, Ayer Hitam and UKM Forest) were evaluated using the total phenol content (TPC), 1, 1-diphenyl-2-picrylhydrazyl free radical (DPPH) and ferric reducing/antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC). Variations in antioxidant activity and total phenolic content in fruit samples among growing locations were much greater than the variation observed between parts of plant (leaves and fruits), indicating that growing locations plays a more important role than parts of plant (leaves and fruits) in *Ficus hispida*. The data indicated that the fruits (Tasik Chini) had the highest total phenolic content (285.42 mg/ 100g DW) and antioxidant activity FRAP, DPPH and ORAC (231.02mg/100g DW, 84.03%, and 84.03µmol/g TE) respectively, while leaves (UKM forest) had the lowest total phenolic content (138.59 mg/ 100g DW) and antioxidant activity FRAP, DPPH and ORAC (126.40 mg/100g DW, 70.87%, and 70.87µmol/g TE) respectively. Correlation analyses indicated that there was a linear relationship between antioxidant activity and the total phenolic content in fruits and leaves. However, growing locations play an important role in the total phenol content and antioxidant activity of *Ficus hispida* leaves and fruits.
Keyword: *Ficus hispida,* growing environment, total phenol content, antioxidant activity.

Introduction

*Ficus*L., commonly known as Fig, is considered as a keystone species in tropical rain forests as it plays very fundamental role in ecosystem, due to its fruits which are eaten by insects, birds and animals throughout the year. The genus *Ficus* represents an important group of trees, not only for their immense value but also for their growth habits and religious value. The genus *Ficus* (Moraceae) comprises about 1200 species distributed mainly in tropical and subtropical regions[1].*Ficus*, is a shrub or small tree, found throughout the year, growing in evergreen forest, moist localities, deciduous forests, to an elevation of 1800 m above sea level, often cultivated in villages for shade and its edible fruits in India, Sri Lanka, Myanmar, southern region of the Republic of China, New Guinea, Australia and Andaman island. Almost all parts of this plant used are bark, leaves, roots, fruits and latex. This genus is characterized by its constituents of coumarins, phytosterols, triterpenes, flavonoids as well as alkaloids and tannins[2-3]. Many *Ficus* species have been used as aphrodisiac, anti hypertensive, anticancer, antioxidant, hepatoprotective, gastroprotective, antidiabetic, anthelmintic, anti malarial, anti-inflammatory, analgesic and antimicrobial [4-7].Antioxidant compounds play an important role in our body due to the positive effect on human health. Consumption of foods containing bioactive compound with

potential antioxidant properties can decrease the risk of human disease such as cancer and heart diseases[8]. Many studies have been made to isolate, characterize and extract antioxidant from natural plant sources. Plant phenolic compounds, and their secondary metabolites flavonoids and proanthocyanidins, have been frequently reported as the active bioactive components associated with antioxidant properties and health benefits[9]. Previous studies have shown that bioactive compounds (tocopherols, sterols, alkylresorcinols, folates, phenolic acids, and fiber components) can be affected by different environmental factors[10]. Although the literature is relatively deficient in this field, [11] showed significant correlations between the contents of bioactive components (alkylresorcinols, sterols, tocols, folates, phenolic acids and fiber compounds) and environmental factors (precipitation and temperature), with even highly heritable components differing in amount between samples grown in different years on different sites. To the best of our knowledge, there have been no reports on the relative contributions of cultivar and region effects of *Ficus* grown in Malaysia, to its bioactive composition, especially phenolics compounds and Antioxidant activity. This study was carried out to determine the effects of growing location on total phenolic content (TPC) and antioxidant activity of *Ficus* leaves and fruits extracts by measuring 2,2-Dipheny-1-picryhydrazyl (DPPH) radical scavenging activity, Ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC).

Materials and Methods

Sample collection

The leaves and fruits of *Ficus hispida* were obtained from four areas (Tasik Chini, Tasik Bera, Ayer Hitam and UKM Forest) Malaysia. The leaves and fruits of *Ficus* were cleaned and cut into small pieces, and then oven dried at 50^{o}C for 24 h. The dried sample was then pulverized using a mechanical grinder and then stored at 4^{o}C until use.

Extraction of antioxidant

In the extraction process, about 1 g of *Ficus hispida* slurries were weighed in universal bottles and 10 ml solvent was added. Solvents used were 50% acetone; samples (*Ficus* slurries with solvents) were then homogenized using homogenizer (T 250, IKA, Germany) at 24,000 rpm for 1 min. All extracted samples were centrifuged by using table top centrifuge (MLX 210, Thermo-line, China) at 4750 g for 10 min. The supernatants were collected for further analysis.

Total phenol content (TPC)

The determination of antioxidant activity through TPC was carried out according to the method of [12]. About 100 Ml *Ficus* extracts was added with 0.5 mL diluted Folin-Ciocalteu reagent. The samples (*Ficus* extracts with Folin-Ciocalteu reagent) were left for 5 min before 1 mL 7.5% sodium carbonate (w/v) was added. The absorbances were taken at 765 nm wavelength with spectrophotometer after 2 hours. Calibration curve of gallic acid was set up to estimate the activity capacity of samples. The result was expressed as mg of gallic acid equivalents per 100 g of dry sample (mg GA/100 g of DW).

Ferric reducing antioxidant power (FRAP)

The determination of antioxidant activity through FRAP was carried out according to the method of [12]. FRAP reagent was prepared fresh as using 300 mM acetate buffer, pH 3.6 (3.1 g sodium acetate trihydrate, plus 16 mL glacial acid made up to 1:1 with distilled water); 10 mM TPTZ (2,4,6-tris (2-pyridyl)-s-triazine), in 40 mM HCLl; and 20 mM FeCl3•6H2O in the ratio of 10:1:1 to give the working reagent. About 1 ml FRAP reagent was added to 100µL *Ficus* extracts and the absorbances were taken at 595 nm wavelength with spectrophotometer after 30 minutes. Calibration curve of Trolox was set up to estimate the activity capacity of samples. The result was expressed as mg of Trolox equivalents per 100 g of dry sample (mg TE/100 g of DW).

DPPH radical scavenging activity

The determination of antioxidant activity through 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging system was carried out according to the method of [12]. Stock solution was prepared by dissolving 40 mg DPPH in 100 ml methanol and kept at -20°C until used. About 350 mL stock solution was mixed with 350 ml methanol to obtain the absorbance 0.01 unit at 517 nm wavelength by using spectrophotometer (Epoch, Biotek, USA). About 100µL *Ficus* extracts with 1 ml methanol DPPH solution prepared were kept for 30min for

scavenging reaction in the dark. Percentage of DPPH scavenging activity was determined as follow: DPPH scavenging activity (%) = $[(A_{blank} - A_{sample}) / A_{blank}] \times 100$. Where A is the absorbance

Oxygen radical absorbance capacity (ORAC)

The ORAC of each *Ficus* leaves and fruits extract as well as AR standards were measured. The ORAC assay was carried out on a fluorescence microplate reader (FLUOstar Omega, BMG LABTECH, Multi-Detection Microplate Reader, Germany). Peroxyl radicals were generated by AAPH, and fluorescence microplate reader was used at an excitation wavelength of 485 nm and an emission wavelength of 525 nm. Trolox was used as standard (50, 25, 12.5, 6.25, 3.12 mM). Proper dilutions of Ficus extracts were made with ORAC buffer (potassium phosphate buffer, pH 7.4). For each ORAC run, a micro plate was prepared containing 25uμ of Trolox standards, buffer control, and sample dilutions, as well as 150ul of fluorescein (FL) solution. All ORAC analyses were performed at 37^0C with a 20 min incubation and 60 min run time. After the incubation, 25ul of AAPH was added to each well for a final volume of 200 uL. The results were calculated using the differences of areas under the FL decay curves between the blank and a sample and were expressed as micromole Trolox Equivalents per gram of sample (umol TE/g) [13].

Statistical analysis

All the analyses were conducted in triplicates for each location. The antioxidant values for the leaves and fruits extracts were evaluated with the one-way ANOVA and Duncan's triplicates Range Test using SPSS software (SPSS ver.19). P values less than 0.05 were considered to be statistically significant. Values were expressed in means ± SD,[14].

Results and Discussion

Total phenolic content (TPC)

Table1 showed significant difference ($P<0.05$) in the total phenolic of *Ficus hispida* leaves and fruits. *Ficus hispida* fruits gave the highest phenolic content (285.42 mg/GAE/100g DW) when compared with *hispida* leaves. The content of phenolic compounds in different growing environment (Tasik Chini, Tasik Bera, Ayer Hitam and UKM Forest) of *Ficus hispida* leaves and fruits is shown in Table 1 with increase in urban area. High content of TPC (285.42 mg/g DW) were obtained from *hispida* fruits in Tasik Chini. After Tasik Chini region, Taski Bera 279.32 mg/100g/ GAE/ DW) had high content of phenolic compounds in extract. In both area (remote and urban), the total phenolic content in the remote areas (Tasik Chini and Taski Bera) were more than the urban areas (Ayer Hitam and UKM Forest). One possible reason for the increased total phenol content and antioxidants activity with the remote areas might be due to the increase in organic matter, rain rate and topography of the land. Our results are different to that reported by [15], where environmental factors were most effective in bioactive compounds.

Table 1:Total phenol content of *F. hispida* leaves and fruits extracts, mg GA/100g DW.

Location	Leaves	Fruits
TasikChini	174.87 ±2.03 [a]	285.42 ± 1.55 [a]
TasikBera	164.70 ± 2.03 [b]	279.32 ± 0.59 [b]
Ayer Hitam	153.51 ± 3.05 [c]	265.07 ± 2.56 [c]
UKM Forest	138.59 ± 0.59 [d]	249.14 ± 1.02 [d]

[a-d] Different letters within the same column indicate significant differences ($P<0.05$).

Ferric reducing antioxidant power (FRAP)

For measurement of the reductive ability, the Fe3+- Fe2+ transformations in the presence of *Ficus hispida* leaves and fruits extracts sample was investigated. Table 2 shows FRAP values for *Ficus hispida* leaves and fruits. The result ranged from (126.40 to164.61 mg /100 g DW) in leaves, (203.03 to 231.02 mg/100 g DW) in fruits. Significant differences ($P<0.05$) in FRAP values were found among the different growing location. Both Tasik Chini area and Tasik Bera area were the best location for finding extracts with higher antioxidant activity. The FRAP value obtained from *hispida* fruits was higher significantly ($P<0.05$) than the extract

obtained from *hispida* leaves. However, for FRAP values sample extracted of *Ficus hispida* leaves and fruits from remot areas (Tasik Chini and Tasik Bera) were significantly (P<0.05) different from urban areas (Ayer Hitam and UKM Forest), also both were significantly (P<0.05) Ayer Hitam higher than UKM Forest for both (leaves and fruits) of *Ficus hispida*. When comparing the results from this study with other study, values from different sources seriously differ. The FRAP mean value in this study showed that leaves and fruits were higher than that of[16].

Table 2: FRAP of *F. hispida* leaves and fruits extracts, mg TE/100g dry weight

Location	Leaves	Fruits
TasikChini	164.61 ± 2.26 [a]	231.02 ± 2.10 [a]
TasikBera	146.41 ± 2.84 [b]	224.61 ± 3.55 [b]
Ayer Hitam	138.56 ± 1.10 [c]	214.39 ± 2.67 [c]
UKM Forest	126.40 ± 2.55 [d]	203.03 ± 1.56 [d]

[a-d] Different letters within the same column indicate significant differences (P<0.05).

DPPH radical scavenging activity

Table 3 shows free radical scavenging activity values of *Ficus hispida* leaves and fruits from different growing environment (Tasik Chini, Tasik Bera, Ayer Hitam and UKM Forest). The results showed that *hispida* fruits is having significantly (P<0.05) higher scavenging activity compared to *hispida* leaves. The results in Table 3 showed that antioxidant activity were sensitive to growing location; generally remote areas (Tasik Chini and Tasik Bera) gave the highest antioxidant activity compare with urban areas (Hitam and UKM Forest). DPPH values of *hispida* leaves and fruits in both areas (remote and urban) decrease with urban area. *Ficus hispida* fruits were found to give the highest values in remote areas. *Ficus hispida* leaves andfruits from Tasik Chini was the best location for obtaining extracts with high antioxidant activates in both leaves and fruits of *hispida* followed significantly (P<0.05) Tasik Bera. The different results obtained from the previous studies may be attributed to different cultivars, growing conditions, maturity stage. [17] also reported that antioxidant properties of fruits and leaves are affected by environment.

Table 3: DPPH *F. hispida* leaves and fruits extracts (%)

Location	Leaves	Fruits
TasikChini	78.02 ± 1.65 [a]	84.03 ±1.35 [a]
TasikBera	76.81 ± 1.44 [b]	82.73 ±1.59 [b]
Ayer Hitam	74.40 ± 1.31 [c]	80.58 ±2.05 [c]
UKM Forest	70.87 ± 2.12 [d]	76.50 ±1.29 [d]

[a-d] Different letters within the same column indicate significant differences (P<0.05).

Oxygen radical absorbance capacity (ORAC)

ORAC measures antioxidant inhibition of peroxyl radical and reflects classical radical chain-breaking antioxidant activity by Hatom transfer [18]. The peroxyl radical generated from thermal decomposition of AAPH in aqueous buffer reacts with a fluorescent probe to form a non-fluorescent product. The effects of growing location (remote and urban areas) in antioxidant activity are shown in Table 4. Significant differences (P<0.05) in ORAC values were found among the different growing environment. As shown in Table 4, significant difference (P<0.05) in ORAC was observed. *Ficus hispida* fruits gave the highest ORAC (88.62µmol TE/g DW) in remote area (Tasik Chini) when compared with urban area (UKM Forest) (54.16 µmol TE/g DW). Comparing antioxidant activity from this study and other published data is difficult due to the fact that content of antioxidant compounds can be influenced by extracting solvent, cultivar and location. [19] reported the ORAC of 25 medicinal plants samples and ORAC values ranged from 2917 to 162 umol TE/g. Also, in another [20]the ORAC values for two different plants varieties were reported between 20 and 37 mmol TE/g. The ORAC-index has previously shown to depend on the target molecule used.

Table 4: ORAC of *F. hispida* leaves and fruits extracts, µmol/ g TE.

Location	Leaves	Fruits
Tasik Chini	65.04 ±1.27[a]	88.62 ±0.27[a]
Tasik Bera	61.04 ± 0.82 [b]	84.49 ± 2.49[b]
Ayer Hitam	57.09 ±3.36[c]	83.81 ±3.85[c]
UKM Forest	54.16 ±1.88 [d]	74.32 ± 3.52[d]

[a-d] Different letters within the same column indicate significant differences (P<0.05).

Correlation of TPC with FRAP, DPPH, and ORAC assays

A correlation analysis among total phenol content (TPC) assays, and antioxidant activity (FRAP, DPPH and ORAC) was performed regardless of the study areas used. A high correlation (Table 5) was found between TPC and antioxidant activity (FPAP, DPPH and ORAC) for both leaves and fruits of *Ficus hispida*. Thus, it can reasonably be concluded that in the extract, antioxidant activity is related to the active component. Findings of researches of correlation analyses among TPC and antioxidant activities are high (Musa et al., 2011).

Table 5: Correlation of the antioxidant activities using different assay

	FRAP	DPPH	ORAC
TPC	0.98	0.77	0.98

Conclusion

The current study investigated the effects of growing location on total phenol content, and antioxidant activity assays of *Ficus hispida* (leaves and fruits) extracts. The results showed that the remote area (Tasik Chini Tasik Bera) has more potential for selected varieties rich in TP content antioxidant activity. The TPC and antioxidant activity (FRAP, DPPH and ORAC) of *Ficus hispida* leaves and fruits extracts were significantly affected by location. *Ficus hispida* used in this study contained highest level of TPC and antioxidant activity, thus a potential source for antioxidants. Besides, all the different growing locations of *Ficus* leaves and fruits are found to be stronger scavenger compared to TPC and antioxidant activity except for the *Ficus*. Despite differences in their storage leaves and fruits, both the parts of plant contained antioxidants that are beneficial to the human body. Hence, the *Ficus* var*hispida* used in this study can be suggested as a suitable source of natural antioxidants.

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