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Original Research Article

Antioxidant Potential and Flavonoid Marker Detection in Two *Ficus carica* Linn. Varieties

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Abstract

Background: Studying the antioxidant properties of various plants used in alternative medicine can support evidence-based medicine practice.
Objectives: This investigation delves into the chemical composition and antioxidant differences of two varieties of *Ficus carica* Linn., red (R) and yellow (Y), cultivated in Malaysia.
Materials and Methods: The leaves were air-dried in shade at room temperature and pulverized into fine powder. Qualitative and quantitative analyses of ethanolic leaf extracts were carried out to observe the phytochemical profiles of the extracts derived from the maceration technique. Utilizing thin-layer chromatography and high-performance liquid chromatography (HPLC), rutin and quercetin were identified, with quercetin identified as the predominant marker. Quantification assays of leaf extracts for both varieties revealed differing phenolic and flavonoid contents. Antioxidant assays, namely, ferric ion-reducing antioxidant power, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays, affirmed the antioxidant activity of both varieties.
Results: Notably, the red variety produced a 23.26% extraction yield, while the yellow counterpart yielded 20.45%, indicating a variance in extraction efficiency. The phenolic compounds and flavonoids, identified through phytochemical screening, denote their potential health benefits. The yellow variety exhibited higher phenolic levels (0.1216 ± 0.014 GAE mg/5 mg), and the red variety possessed elevated flavonoid content (0.189 ± 0.021 QE mg/5 mg), with the yellow variety demonstrating superior efficacy.
Conclusion: These findings emphasize the significance of extraction methodologies and variety selection in optimizing the antioxidant potential of *F. carica* L. extracts, offering promising prospects for their integration into health-focused functional food products.

Keywords

Ficus carica L., antioxidant activity, phenolics, phytochemical screening, flavonoids, chromatography

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Introduction

The *Ficus* genus is part of the Moraceae plant family, representing a significant and diverse group within the angiosperms, encompassing over 800 species. These plant species manifest in various forms, such as trees, shrubs, hemiepiphytes, climbers, and creepers, and are predominantly located within subtropical and tropical regions worldwide (Mawa et al., 2013). The genus *Ficus* provides substantial resources due to its economic and nutritional value and plays a key role in preserving rainforest biodiversity. The plants are major food source for many fruit-eating animals in tropical regions (Rønsted et al., 2007). The genus is categorized into six subgenera, according to morphology: *Sycomorus* (Gasp.) Miq, *Synoecia (Miq.) Miq*, *Sycidium* (*Miq.) Mildbr.* and *Burret*,

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Ficus (gyno-dioecious), *Pharmacosycea (Miq.) Miq.* (monoecious), and *Urostigma (Gasp.) Miq*. (Ramesh et al., 2021). *Ficus carica* L. is prized for its delicious fruits and is employed in culinary delights and traditional medicine. Its distinctive lobed leaves and unique syconium fruit structure characterize it morphologically. Other prominent *Ficus* siblings include *Ficus benghalensis* (Banyan Tree), esteemed for its ornamental value and the aerial roots that adorn its expansive canopy; *Ficus elastica* (Rubber Fig), favored as an indoor plant and historically for rubber production, with its broad, glossy leaves; *Ficus lyrata* (Fiddle-leaf Fig), renowned for its large, fiddle-shaped leaves, adding a tropical ambiance to interiors; and *Ficus benjamina* (Weeping Fig), cherished for its graceful, weeping foliage, ideal for both indoor and outdoor settings. These species not only contribute to horticulture but also offer health benefits that could potentially enhance their value for human well-being, including antioxidant and anti-inflammatory effects.

F. carica L., commonly referred to as a fig tree, originates from the Mediterranean region and Southwest Asia. This deciduous tree is among the earliest cultivated plants. It typically reaches a height of approximately 15–20 ft and is characterized by its wide branching structure. The trunk of this tree seldom exceeds a diameter of 7 ft (Badgujar et al., 2014). The skin of the fruit produced by this tree is notably thin and delicate in its fresh state. It is a rich source of minerals, fibers, sugars, vitamins, and organic acids, and notably, it contains phenolic compounds. The succulence of the pulp is complemented by a strikingly diverse color range. This spectrum of colors, ranging from whitish and pale-yellow tones to deeper hues of pink, rose, red, or purple, is not merely aesthetic but suggests a complex interplay of genetic and environmental factors inherent to each specific cultivar of the tree species (Isa et al., 2020). The variation in peel coloration is mainly due to anthocyanin accumulation, with the type and concentration of these pigments differing among cultivars (Dueñas et al., 2008). The diversity in the coloration of the fruit potentially reflects varying phytochemical compositions, which can lead to different potential therapeutic uses.

F. carica L. has been historically utilized for its therapeutic properties in addressing a range of health concerns. It is used in Ayurvedic, Unani, and Siddha traditional medicine to address gastric problems, inflammation, and cancer (Mawa et al., 2013; Rasool et al., 2023), whereby different parts of the plant, such as leaves, fruits, and roots, are employed to treat a multitude of disorders. Historically, it has been acclaimed for its therapeutic properties, serving as a natural laxative and offering respiratory, cardiovascular, anti-inflammatory, and anti-spasmodic benefits (Guarrera et al., 2005). In the gastrointestinal tract, it addresses conditions such as indigestion, colic, diarrhea, and loss of appetite. In traditional Indian medicine, these fruits are commonly employed for their laxative properties, as well as for their expectorant and diuretic effects (Mawa et al., 2013). In Malaysia, Mawa et al. (2013) reported that the ethnopharmacological uses of *F. carica* L. are traditionally for treating respiratory ailments, specifically for coughs and tuberculosis. However, it is important to note that not all traditional uses of this plant have been thoroughly researched, and further investigation is needed to substantiate these claims and understand their efficacy and safety.

F. carica L. is rich in phenolic compounds with various physiological functions within the plant system. The pharmacological efficacy of the fig tree is primarily attributed to its rich array of secondary metabolites, encompassing bioactive compounds like flavonoids, polyphenols, tannins, coumarins, organic acids, carotenoids, and vitamin E (Bucić-Kojić et al., 2011). These compounds are also beneficial to human health, primarily due to their multifaceted antioxidant capabilities. These benefits include functioning as reducing agents, providing hydrogen, scavenging free radicals, and quenching singlet oxygen. These compounds are recognized for their ability to counter oxidative stress, thereby preventing degenerative conditions such as cancer, cardiovascular diseases, diabetes, and neurodegenerative disorders, including Parkinson's and Alzheimer's disease (Hasnain et al., 2023). Among these bioactive compounds, flavonoids are a diverse group of over 9,000 compounds present in fruits and medicinal plants and are known for their antioxidant, anti-inflammatory, and anti-cancer properties (Liu et al., 2021; Ramesh et al., 2021; Zhang et al., 2020). Notable among these are flavonoids, specifically luteolin and quercetin, which are the most abundant compounds found in the leaf extract (Khan et al., 2017; Mota et al., 2009; Sabiu et al., 2016; Vaya & Mahmood, 2006).

In recent years, numerous studies have further supported the therapeutic role of flavonoid-rich plants in health and disease. For example, quercetin and rutin have been widely explored for their anti-oxidative, anti-inflammatory, and cardiometabolic benefits. Meta-analyses have reported significant reductions in systolic blood pressure, low-density lipoprotein (LDL) cholesterol, and fasting glucose levels with quercetin supplementation (Chen et al., 2022; Li, Yao, et al., 2021). Additionally, flavonoid-rich diets are associated with improved vascular function and reduced oxidative stress (Singh et al., 2020). Beyond antioxidant activity, *F. carica* L. extracts have also demonstrated anti-microbial, anti-diabetic, and anti-obesogenic effects, particularly through mechanisms such as enzyme inhibition and modulation of glucose and lipid metabolism (Ayoub et al., 2019; Kebal et al., 2022). These findings reinforce the broader potential of *F. carica* L. as a functional food and nutraceutical agent.

Recent systematic reviews have reinforced the multifunctional health benefits of flavonoid-rich plants like *F. carica* L. beyond antioxidant properties. Rasool et al. (2023) emphasized the industrial and pharmacological potential of fig by-products, citing their anti-cancer, anti-ulcer, and anti-diabetic properties supported by both *in vitro* and *in vivo* studies. Similarly, Hasnain et al. (2023) reviewed the immunomodulatory, cardioprotective, and respiratory benefits of fig leaves, highlighting their phenolic and flavonoid

constituents as key therapeutic agents. These findings support the use of *F. carica* in functional foods and nutraceutical development aimed at addressing chronic metabolic and inflammatory disorders.

Despite existing research on the antioxidant properties of *F. carica* L., there is limited comparative analysis between red and yellow *F. carica* varieties regarding their phytochemical composition and antioxidant capacity. We hypothesize that these varieties exhibit distinct phytochemical compositions and antioxidant potentials. Specifically, we predict higher levels of flavonoids, such as quercetin and rutin, in the red variety compared to the yellow variety. Notably, studies by Alshaal et al. (2020) and Li, Yu, et al. (2021) have consistently identified and quantified these compounds in *F. carica* L. extracts. Furthermore, Li, Yu, et al. (2021) highlighted three flavonoids with remarkable antioxidant capacity, including isoschaftoside, 3-*O*-(rhamnopyranosyl-glucopyranosyl)-7-*O*-(glucopyranosyl)-quercetin, and rutin. These findings not only validate our choice of chemical markers but also highlight their significance in the plant's medicinal properties. Incorporating the mentioned flavonoids as markers can be regarded as a reference to our research findings, which corresponds to the scientifically supported approach. This study sought to identify the extraction yield, as well as quercetin and rutin contents in the extracts obtained using the maceration technique, thin layer chromatography (TLC), and high-performance liquid chromatography (HPLC), which are standardized, valid methods, in *F. carica* L. plants cultivated in Malaysia. Furthermore, the current research investigates the complex chemical compositions of the leaves and evaluates their antioxidant potential using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. In addition to the above antioxidant tests, the leaf extracts were complemented by quantifying the total flavonoid and phenolic content.

Therefore, this study offers a new outlook into the phytochemical profile and the antioxidant capacity of red and yellow varieties of *F. carica* L. cultivated in Malaysia. Although some previous studies have focused on the antioxidant activity of *F. carica* L., a systematic analysis of the differences between the two varieties has not been explored. In addition, due to differences in cultivating environments, exploring these compounds and their antioxidant activity in a local context is important. This study seeks to fill this gap by presuming that the phytochemicals in the red and yellow varieties differ and that they have dissimilar antioxidant capacities. By employing techniques such as TLC, HPLC, DPPH, and FRAP, the study also aims to identify the specific compounds that are responsible for these properties. In addition, this study will establish the total flavonoid and phenolic content, hence adding to the quantity of information on the health benefits of *F. carica* L. The methodology not only helps expand our knowledge of this species but also has benefits that can be applied in other fields, such as the pharmaceutical and functional foods industry.

Materials and Methods

Materials

The following instruments and consumables were used in this study: an analytical balance (Mettler Toledo AT201), an electric mill grinder (Retsch SM100), a Buchi rotary evaporator (R124/A), Whatman no. 1 filter paper, a UV detection box with 254 and 366 nm lamps, TLC plates utilizing silica gel 60 F254 (from Merck, Germany), a 96-well plate, a vortex mixer (Model VM-10), a microplate reader (VersaMax™), and HPLC (Shimadzu, HPLC-LC20AT) with a Diode Array Detector (SPD-M20A). The chemicals utilized were HPLC-grade acetonitrile (Fisher ChemAlert®), methanol (Chemiz), ethanol (HMbG®), ethyl acetate (Chemiz), and chloroform (LiChrosolv®). The standard samples of rutin and quercetin were from Merck, Darmstadt, Germany.

Plant Material Identification and Preparation of F. carica L. Leaves Extract

F. carica L. leaves from yellow and red fruit varieties were collected from Muallim Hortigard and Nursery in Perak, Malaysia. They were identified by Dr. Yong Kien Thai, a plant taxonomist at Rimba Ilmu Botanic Garden, Universiti Malaya (UM). To facilitate future reference, voucher specimens, distinguished by identification numbers KLU50204 (yellow) and KLU50203 (red), have been systematically cataloged and stored within the herbarium archives of Rimba Ilmu, UM. These leaves were air-dried in the shade at room temperature (30°C ± 2°C) for 2 weeks to preserve their natural state, pulverized into fine powder utilizing an electric mill grinder, and stored in airtight containers. For the extraction, 500 g of the powdered material from each type of leaf was soaked in absolute ethanol (1,500 mL) for 5 days, based on a modified method from Nurlyana et al. (2018). The ethanolic leaf extract (ELE) was then filtered by vacuum filtration utilizing Whatman no. 1 filter paper, further processed, and condensed under decreased pressure at 45°C in a Buchi rotavapor (R124/A). The resulting extract, as shown in Table 1, was stored at 4°C in the dark.

Phytochemical Profiling

To achieve the goal of this study, the following tests were used to identify the flavonoid content of the *F. carica* L. plant. Phytochemical analysis and TLC were used as preliminary tests, whereas HPLC was used as a confirmatory test. The following sections provide details about each of these tests.

Preliminary Phytochemical Screening

A series of chemical tests was performed on the ELE to ascertain the presence or absence of specific phytochemical entities within the extract. An aliquot test sample of the ethanolic

extract was taken in a test tube, and the specific chemical reagents to detect various phytoconstituents were added dropwise. Subsequently, a chemical reaction and a color change, corresponding to the class of phytoconstituents, were observed and recorded, especially for flavonoids, phenolics, and alkaloids, as illustrated by Alsadi et al. (2022). The outcome of these tests provides valuable insights into the phytochemical profile of ELE, as demonstrated in Table 2.

Test for Flavonoids

Alkaline test: The crude plant extract solution was treated with 4–5 drops of 10% ammonium hydroxide.

Test for Phenolic Compounds

Ferric chloride test: 5 mL of the crude plant extract solution was mixed with 1 mL of a 1% gelatin solution incorporated with sodium chloride and shaken well. The appearance of a bluish-black/greenish-black color signified the presence of phenols.

Gelatin test: About 5 mL of the crude extract solution (0.05 g of extract in 5 mL distilled water) was combined with approximately 3 mL of a 1% gelatin solution, vigorously shaken, yielding a white precipitate formation, suggesting the presence of phenols.

Test for Alkaloids

Wagner's test: 2 mL of Wagner's reagent (consisting of iodine [1.27 g] and potassium iodide [2 g]) was dissolved in 5 mL of water and diluted to 100 mL, and was added to 1 mL of the extract diluted with hydrochloric acid. The observation of a reddish-brown precipitate indicates the presence of alkaloids.

Thin Layer Chromatography of ELE

Rigorous adherence to established TLC protocols involved dissolving 1 mg of crude ethanolic extracts and the standards (rutin and quercetin) in solvents and applying them to Silica gel 60 plates. The extracts were spotted onto TLC plates with

Table 1. The Initial and Final Weights of Yellow/Red Varieties of *Ficus carica* L. and Antioxidant Assays.

	TEY%			TWL%			TPC	TFC	FRAP	DPPH
F. carica L. Variety	Initial Weight (Powder)	Weight of Extract	Extraction Yield (%)	Wet Weight of Leaf	Dry Weight of Leaf	% Moisture Loss	(mg GAE/g ± SD)	(mg QE/g ± SD)	(mg $FeSO_4$/mg ± SD)	IC_{50} (mg/mL ± SD)
Yellow	500 g	102.23 g	20.45	5,000 g	1,011 g	79.78	0.1216 ± 0.014	0.105 ± 0.007	0.351 ± 0.014	0.651 ± 0.298
Red	500 g	116.32 g	23.26	4,500 g	914 g	79.69	0.0858 ± 0.021	0.189 ± 0.021	0.237 ± 0.067	1.123 ± 0.037

Note: DPPH: 2,2-Diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing antioxidant power; GAE: Gallic acid equivalent; TEY: Total extraction yield; TFC: Total flavonoid content; TPC: Total phenolic content; TWL: Total weight loss.

Table 2. Phytochemicals Screening and Thin Layer Chromatography (TLC) Profiling of Ethanolic Leaf Extract (ELE) of *Ficus carica* L. Varieties in Comparison to Standards like Rutin and Quercetin.

Phytochemical Screening Result				TLC Result					
Constituents	Test	Red	Yellow	Dilution Solvent Standard/Extract	Mobile Phase	Y R_f	R R_f	SR R_f	SQ R_f
Alkaloids	Wagner's	–	–	Ethanol	Ethyl acetate:Methanol: Water (8:1:1)	0.39	0.39	0.40	–
Phenolic compounds	Ferric chloride	+++	+++	Ethanol	Ethyl acetate:Chloroform: Water (5.5:4:0.5)	0.71	0.71	–	0.71
	Gelatin	++	++						
Flavonoid	Alkaline reagent	++	++						

Notes: (+ ++), high presence; (+ +), moderate presence; (+), low presence; (–), not detected. R: Red variety; SQ: Standard quercetin; SR: Standard rutin; Y: Yellow variety.

the help of a capillary tube. Different and suitable mobile phases (methanol, water, chloroform, and ethyl acetate) were employed to obtain the best separation medium for flavonoids. Components were visualized using UV light at wavelengths of 366 and 254 nm. R_f values, indicating a compound's affinity with the mobile phase, were calculated using the formula:

$$Rf = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent front}}$$

These R_f values can be found in Table 2 and are crucial for compound identification and comparison in chromatographic separations.

Detection of Rutin and Quercetin in ELE by HPLC-PDA

Adhering to the methodology established by Alshaal et al. (2020) with some modifications, the study employed an HPLC system comprised of a 4050 pump, column oven, and 2850 photodiode array (PDA) detector. The chromatographic analysis was conducted using a Eurospher C18 column (250 × 4.6 mm ID, 5 µm particle size) for the detection of quercetin and rutin in the *F. carica* L. leaf extracts. The method followed published validation parameters (Alshaal et al., 2020), and the limits of detection (LOD) and limits of quantification (LOQ) were not determined in this study. The separation process utilized a mobile phase gradient consisting of solvent A (water) and solvent B (acetonitrile with 0.3% acetic acid). The gradient elution was executed under the following conditions: (0–2 min, 100% A), (2–12 min, gradient to 70% A), (12–14 min, 70% A to 35% A), and (14–17 min, 100% A), at a flow rate of 0.9 mL/min. The injection volume for each analysis was set at 20 µL, with each sample undergoing triplicate injections. A PDA detector was used to monitor the UV–visible absorption spectrum within a range of 200–600 nm, specifically focusing on a wavelength of 370 nm for the detection of quercetin and rutin.

Total Phenolic Content (TPC)

The Folin–Ciocalteu (F–C) phenol method was applied for the quantification of the TPC of *F. carica* L. leaf extracts, according to the method described by Afrin et al. (2023), with a slight modification. Methanol was used to solubilize the extracts and the standard. In short, 20 µL (1 mg/mL) aliquots of the samples were mixed with 50 µL of F–C reagents (diluted with distilled water at a ratio of 1:10) in the 96-well plate, and then 100 µL of Na_2CO_3 (60 g/L) was added. The plate was then incubated for 60 min at room temperature, and the absorbance at 750 nm was measured using a microplate reader (VersaMax™). The calibration curve was prepared using gallic acid as a standard (0.0098, 0.0195, 0.0390, 0.0781, 0.1563, and 0.3125 mg/mL; r^2 = 0.9731), and the TPC values were expressed as mg GAE (gallic acid equivalent)/mg of dry extract.

Total Flavonoid Content (TFC)

The quantification of the TFC in the extracts, derived from *F. carica* L. leaves, was conducted following the methodology delineated by Afrin et al. (2023). Methanol was used to solubilize the extracts and the standard. In brief, 100 µL (1 mg/mL) aliquots of the samples were mixed with 100 µL of a 2% w/v solution of aluminum chloride (0.2 g in 10 mL of methanol) in the 96-well plate. The plate was then incubated for an hour at room temperature, and the absorbance at 430 nm was measured using a microplate reader (VersaMax™). The calibration curve was prepared using quercetin as a standard (0.0098, 0.0195, 0.0390, 0.0781, and 0.3125 mg/mL; r^2 = 0.9999), and the TFC values were expressed as mg QE (quercetin equivalent)/mg of dry extract.

In Vitro Antioxidant Activities

DPPH Assay

The DPPH assay was performed according to the procedure outlined by Afrin et al. (2023). Initially, 5 mg of *F. carica* L. extracts were dissolved in 1 mL of methanol to create stock solutions. These stock solutions were subsequently diluted to five different concentrations (0.025, 0.05, 0.1, 0.25, and 0.5 mg/mL) using methanol. Each concentration was tested in triplicate (n = 3). The samples were thoroughly mixed using a vortex mixer for 1 min. Next, 100 µL of each sample concentration was mixed with 60 µL of DPPH solution (0.2 mg/mL in methanol) in the 96-well plate. The plate was then incubated at room temperature for 30 min, and the absorbance at 517 nm was measured using a microplate reader (VersaMax™). Methanol served as the blank, and the percentage of radical scavenging activity (RSA) was calculated using the following equation:

$$\%\text{RSA} = \left[\text{Absorbance}_{\text{blank}} - \text{Absorbance}_{\text{sample}}\right] / \text{Absorbance}_{\text{blank}} \times 100$$

The percentages obtained from the assay at different concentrations were plotted on a graph, and the results are presented as IC_{50} values in mg/mL. The IC_{50} represents the concentration of *F. carica* L. samples required to inhibit 50% of free radicals. Gallic acid was employed as a reference compound in the experiment at a concentration of 0.1 mg/mL and analyzed under the same conditions as the sample extracts.

FRAP Assay

The FRAP assay was performed following the procedure detailed by Kebal et al. (2022) with a slight modification. The FRAP reagents were prepared with the ratio 10:1:1 of solution A (300 mM acetate buffer), solution B (10 mM TPTZ),

and solution C (20 mM $FeCl_3 \cdot 6H_2O$). In summary, 10 µL aliquots of 1 mg/mL extract concentration were mixed with 300 µL of FRAP reagents in the 96-well plate. Each assay was performed in triplicate ($n = 3$). The plate was then incubated for 4 min at room temperature, and the absorbance at 470 nm was measured using a microplate reader (VersaMax™). The calibration curve was prepared using iron sulfate ($FeSO_4$) as a standard (0.0098, 0.0781, 0.1563, 2.5000, and 5.0000 mg/mL; $r^2 = 0.972$), and the FRAP values were expressed as mg iron sulfate ($FeSO_4$) equivalent/mg of dry extract.

Statistical Analysis

Data was analyzed using Excel software. Continuous variables were presented as mean ± standard deviation (SD), and categorical variables were presented as proportions. The retardation factor (RF), RSA, and extraction yield were calculated by specific formulae as aforementioned in the corresponding sections.

Results

F. carica L. is commonly known as "Buah tin" in the local Malay language (Milow et al., 2013), "Teen" in Arabic, and "fig" in English (Khan, 2018). There are distinct varieties of this plant characterized by notable differences in leaf morphology and fruit color. The red and yellow varieties are popular for their health benefits. The leaf of the red variety is characterized by a typical lanceolate shape, with irregular margins contributing to its unique appearance. The leaf of the yellow variety is characterized by broader, ovate, and smoother edges. Beyond their leaf morphology, the most visible disparity between these two varieties lies in the coloration of their fruits. The red variety displays a rich, crimson hue, reminiscent of ripe cherries. The surface of the fruit is smooth and glossy, and it has succulent flesh. The yellow variety offers thick skin like the red variety, with a stark contrast of golden-yellow coloration, as shown in Figure 1. In addition to morphological differences, these two varieties offer distinct phytochemical profiles, which may contribute to their antioxidant capabilities.

The objective of this study was to distinguish between the two varieties based on phytoconstituents, particularly flavonoids like quercetin and rutin, as they are the primary free radical scavenging molecules, and to determine their antioxidant activity. This research was carried out in two phases, where the initial phase involved the qualitative analysis of the ELE of *F. carica* L. varieties using TLC, HPLC, and

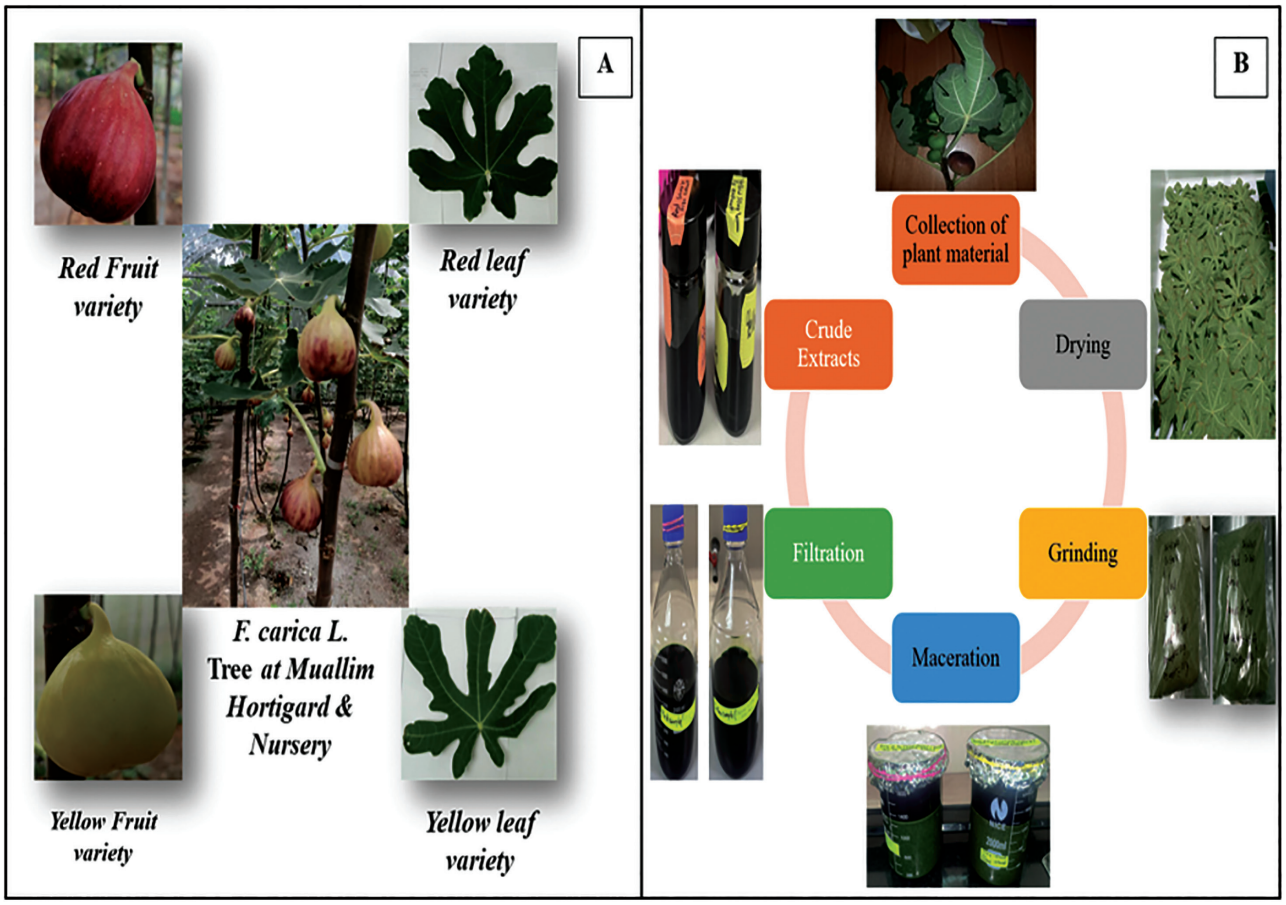


Figure 1. (A) A Morphological Description of *Ficus carica* L. Varieties (B) Extraction Process of *F. carica* L. Leaf.

phytochemical tests, followed by quantitative analysis for phenolics, flavonoids, and antioxidant assays.

Percentage of Total Extraction Yield (TEY) and Total Weight Loss (TWL)

As shown in Table 1, there was a minor variation in the percentage of weight loss post-drying between the yellow and red varieties, with values of 79.78% and 79.69%, respectively. However, a significant variation in the yield of yellow and red varieties was observed after ethanolic extraction, with values of 20.45% and 23.26%, respectively, as indicated in Table 1. However, according to a study by Kebal et al. (2022), the black peel fig variety yielded 11.46% extract, while the red peel fig variety yielded 10.68% extract, both based on dry weight. Our study observed a higher yield from red fig varieties compared to yellow varieties after ethanolic extraction. The rationale for selecting ethanol as a solvent of choice for phenolic extraction is due to its inherent safety for human consumption and versatile solvent characteristics. Previous research conducted by Trifunschi and Ardelean (2013) demonstrated the efficacy of ethanol in extracting bioactive compounds, as evidenced by the notably higher extraction yields achieved with ethanol (70%) compared to distilled water across various extraction methods, including maceration, microwave-assisted extraction, and stirring. Additionally, research performed by Radwan et al. (2020) showed that *F. carica* L. extraction with methanol (100%) and ethanol (95%) yielded the highest at approximately 23%. Increasing the volume content of ethanol or methanol led to higher yields. In contrast, water extraction yielded only about 8%. Moreover, ethanol with moderate polarity facilitates the dissolution of a wide range of molecular species due to its intermediate nature between polar and non-polar solvents. Consequently, ethanol serves as an effective medium for the extraction of diverse constituents, lipids, carbohydrates, proteins, pigmented compounds, and phytochemicals, which are prevalent in species such as *F. carica* L. (Liu et al., 2021). Therefore, ethanol was preferred for the efficacious separation of phytochemicals from *F. carica* L.

Phytochemical Screening

The comparative analysis of phytochemical components in red and yellow leaf extracts of *F. carica* L., as depicted in Table 2, reveals significant similarities in their qualitative phytochemical profiles. Both extracts predominantly contain phenolic compounds and flavonoids while being notably deficient in alkaloids. The above observation aligned with the findings of Shad et al. (2014) and Sharma et al. (2017), who also identified similar phytoconstituents in different solvent extracts of *F. carica* L. In particular, the ethanol extracts showed the highest concentration of these phytochemicals, a finding echoed by Kebal et al. (2022). The presence of these phytochemicals, especially flavonoids, underlines their potential in health enhancement and medicinal applications due to their anti-mutagenic, anti-inflammatory, anti-microbial, and antioxidant properties. Traditional medicine acknowledges the miraculous benefits of thousands of plant species, including those within the *Ficus* genus. The leaf and bark decoction of *Ficus bengalensis*, *Ficus racemosa*, *Ficus hispida*, and *Ficus lacor* have been utilized in Asian traditional remedies for diabetes (Khan et al., 2011). Furthermore, the fresh leaves of the plant are used as a blood tonic, aiding in iron supplementation and combating conditions such as diarrhea, anemia, and sexually transmitted diseases in Africa (Murkute et al., 2022). Chinese folk medicine utilizes *Ficus microcarpa* for the treatment of headaches, toothaches, liver issues, foot inflammation, rheumatism, and wounds.

Thin-layer Chromatography to Detect Rutin and Quercetin in the ELE

In the TLC assessment of *F. carica* L. extracts, the analysis of comparable R_f values with rutin and quercetin standards in both yellow and red varieties suggests analogous phytochemical profiles. Ethanol was used to dilute ELE, and then they were subjected to TLC separation under various mobile phases, as shown in Table 2. These findings align with the R_f range (0.39–0.71) of standard compounds rutin (SR) and quercetin (SQ), indicating the presence of these flavonoids in the ELE. Interestingly, the quercetin standard and ELE exhibited R_f values of 0.71 under both UV wavelengths, consistent with previous findings by Dewi et al. (2023), which reported similar R_f values for quercetin under comparable conditions. Sharma et al. (2017) indicated the presence of quercetin and rutin in the analyzed ethanolic extracts in their study. Phytochemicals exhibited distinctive R_f values across various mobile phases, shedding light on the differential migration of compounds under assorted chromatographic conditions. These results offered a comparative profile of yellow and red *F. carica* L. varieties in contrast to rutin and quercetin.

Detection of Rutin and Quercetin in ELE Using HPLC-PDA Analysis

In the preliminary TLC, results of ELE hinted at the presence of markers such as quercetin and rutin. However, to confirm the presence of these markers, we employed the advanced precision of HPLC. Modern techniques, such as HPLC, are pivotal in standardizing herbal extracts. It quantifies active compounds, ensuring consistent product quality. By identifying constituents, it helps control therapeutic benefits. HPLC also detects contaminants, which is crucial for product safety and regulatory compliance (Murkute et al., 2022). Its role extends to validating herbal monographs and aiding research for new products. Overall, the ability of HPLC to provide accurate quantitative data, identify active compounds, ensure quality control, and detect contaminants underscores its necessity and significance in meeting the regulatory standards

set by organizations such as the WHO, OECD, and FDA. The purpose of this study was to detect the presence of rutin and quercetin, key marker compounds in ELE, and to develop a simultaneous analytical detection method for these compounds. The results, as shown in Figure 2, show that rutin and quercetin in *F. carica* L. leaves were found at retention times of 15.76 min and 22.54 min, respectively. Furthermore, it was noticed that the quercetin peak area was higher than that of rutin in both red and yellow leaf samples. Moreover, while Kebal et al. (2022) identified rutin as the predominant flavonoid in *F. carica* L., the current study revealed a higher dominance of quercetin. According to Vaya and Mahmood (2006), the primary flavonoids within *F. carica* L. are quercetin and luteolin, with concentrations of 631 and 681 mg/kg extract, respectively. In fact, rutin, structurally composed of quercetin and rutinose, undergoes metabolism where it is hydrolysed into quercetin and rutinose (Yang et al., 2019), allowing the body to benefit from the antioxidant properties of quercetin. The pharmacological and nutritional significance of these compounds is correlated with powerful antioxidant properties with health benefits, including cardiovascular protection, immune modulation, and potential anti-cancer effects. The impact of varying experimental conditions in HPLC analyses, such as flow ratios and sample preparation techniques, on the quantification and identification of flavonoids is critical. Further analytical method development is necessary to shorten the retention time in the detection of rutin and quercetin.

Estimation of TPC and TFC and Antioxidant Assays

TPC and TFC Assay

TPC and TFC are chemical assays used to quantify the concentration of flavonoids and phenolic compounds in a sample, respectively. TFC, detected by the F–C phenol method, relies on the reduction of the F–C reagent by flavonoids under alkaline conditions, forming a blue-colored complex that is measured spectrophotometrically. In contrast, TPC, based on the aluminum chloride solution method, involves the reaction of phenolic compounds with aluminum chloride under acidic conditions, resulting in the formation of a yellow complex, which is also measured spectrophotometrically. Both assays utilize specific chemical reactions to quantify the target compounds and provide valuable insights into the antioxidant capacity and potential health benefits of various plant-based materials, including herbs, fruits, vegetables, and beverages. The results in Table 1 show that the yellow variety has a higher TPC (0.1216 ± 0.014 GAE mg/1 mg) than the red variety (0.0858 ± 0.021 GAE mg/1 mg), suggesting a richer phenolic composition. Conversely, the red variety exhibited a

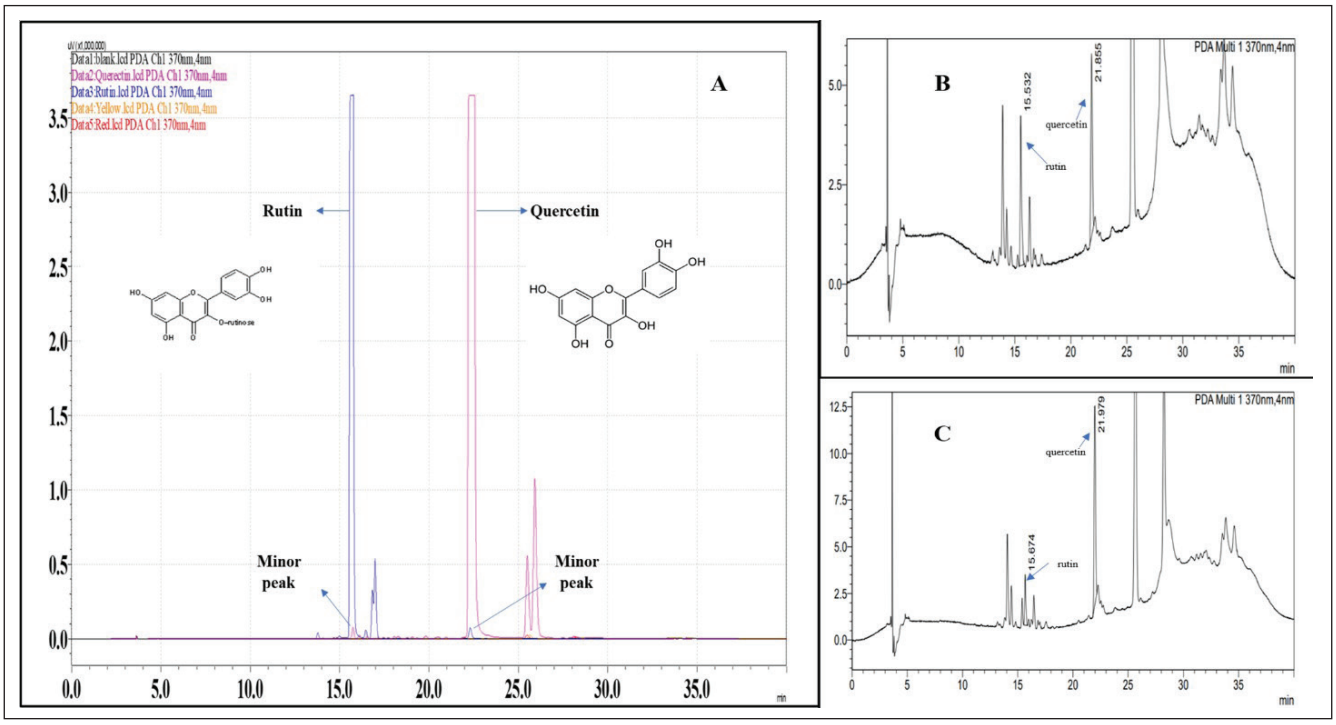


Figure 2. High-performance Liquid Chromatography with Photodiode Array Detection (HPLC-PDA) Chromatograms of Ethanolic Leaf Extract (ELE) and Simultaneous Detection of Rutin and Quercetin Markers (A) Chromatogram with Rutin and Quercetin at 15.76 min and 22.54 min Respectively (B) Chromatogram of ELE Red Variety with Rutin and Quercetin (C) Chromatogram of ELE Yellow Variety Rutin and Quercetin. Flow Rate: 0.9 mL/min, Wavelength: 370 nm, Using a C_{18} Column (250 mm × 4.5 mm ID, 5 µm) with Gradient Mobile Phase (A: Water and B: Acetonitrile).

greater TFC (0.189 ± 0.021 QE mg/1 mg) compared to the yellow (0.105 ± 0.007 QE mg/1 mg). As noted by Bey and Louaileche (2015), dark-colored fruits (like red) tend to exhibit higher flavonoid content compared to light ones (like yellow), consistent with our findings. Interestingly, this contradicts the common observation that dark-skinned fruits generally exhibit higher polyphenol contents compared to light-skinned ones, as mentioned by Khadhraoui et al. (2019). Galeotti et al. (2018) noted that the quantitative presence of phenolics and flavonoids within the extract constitutes its anti-oxidative potency. A similar study by Pratami et al. (2018) revealed a consistent association between elevated TPC and TFC levels and significant antioxidant activity. Flavonoids as antioxidants demonstrate various pharmacological potentials, including anti-hepatotoxic, anti-tumor, anti-ulcer, cardiovascular, diabetes, and infections (Chang et al., 2016; Wang et al., 2018). The results indicate that both varieties of *F. carica* L. exhibited antioxidant activity, as reflected by their respective levels of TPC and TFC, suggesting their bioactivity potential. These findings underscore potential variances attributable to genetic, environmental, or methodological factors and highlight the necessity for further in-depth research to explore the unique health-promoting characteristics inherent in each variety.

FRAP Measurement

The FRAP test provides valuable information about the total antioxidant capacity of various samples, including foods, beverages, and biological fluids, and is widely used in nutritional and biomedical research to assess the potential health-promoting properties of antioxidants. The FRAP test is a chemical assay utilized to measure the antioxidant capacity of a sample. This assay depends on the capacity of antioxidants in the sample to convert ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) in an acidic environment, leading to the formation of a blue-colored ferrous-tripyridyl triazine complex (Payne et al., 2013). The intensity of the blue formed is measured spectrophotometrically at a 470 nm wavelength, with a higher absorbance indicating a greater antioxidant capacity of the sample.

The study findings revealed significant variations in antioxidant capacities among red and yellow varieties. Specifically, the ELE of red exhibited an antioxidant value of 0.237 ± 0.067 mmol Fe (II)/1 mg, in contrast to the yellow fig extract, which demonstrated a notably higher value of 0.351 ± 0.014 mmol Fe (II)/1 mg (Table 1). According to Hssaini et al. (2020), dark-colored fruits (like red) demonstrated higher ferric-reducing abilities, while lighter-colored fruits (like yellow) exhibited lower levels of ferric-reducing activity. Although Kebal et al. (2022) reported that the aqueous extract of the black peel variety had a value of 30.00 ± 3.25 mmol Fe (II)/100 g of lyophilized extract, the red peel variety extract had a value of 18.87 ± 2.62 mmol Fe (II)/100 g of lyophilized extract. In contrast, our results showed that ELE of lighter-colored varieties displayed higher FRAP values. Growing conditions and genetic differences among the varieties could contribute to these variations. In the study conducted by Ayoub et al. (2019), the antioxidant potency of *F. carica* L. of unspecified leaves showed a dose-dependent FRAP activity. This robust antioxidant activity was linked to the rich presence of secondary metabolites like alkaloids, flavonoids, and polyphenols. The results indicated significant capabilities in scavenging free radicals, inhibiting lipid peroxidation, and preventing DNA damage, which may potentially mitigate carcinogenesis processes. These findings align with the results of our study, indicating higher antioxidant activity for ELE of both red and yellow varieties, indicating the presence of substantial concentrations of phenolic and flavonoid compounds. Nevertheless, the extraction techniques can affect the antioxidant potential of botanical extracts. According to Bucić-Kojić et al. (2011), ethanolic extraction increases the TFC/TPC ratio with a more efficient extraction process. Furthermore, using different extraction methods, such as maceration and ultrasound-assisted extraction (UAE), can result in varying levels of TPC/TFC contributing to antioxidant activity (Shahinuzzaman et al., 2020). Therefore, standardized methodologies should be employed to understand the relationship between antioxidant capabilities and extraction techniques.

The 2,2-Diphenyl-1-Picrylhydrazyl Measurement

The DPPH assay is a chemical method employed to evaluate the antioxidant activity of a sample. In this assay, the DPPH radical, a stable free radical with a deep purple color, reacts with antioxidants present in the sample, resulting in the reduction of DPPH and a corresponding decrease in its absorbance. The extent of discoloration, measured spectrophotometrically at 517 nm, is indicative of the scavenging ability of the sample's antioxidants. A higher percentage of DPPH radical scavenging indicates a greater antioxidant capacity of the sample. The antioxidant activity observed in the DPPH assay is primarily attributed to the ability of phenolic compounds to neutralize free radicals via two principal mechanisms: hydrogen atom transfer and single-electron transfer. In the hydrogen atom transfer mechanism, phenolic hydroxyl groups donate hydrogen atoms to the DPPH radical, producing the non-radical DPPH-H form. In the single-electron transfer mechanism, phenolics donate an electron to the DPPH radical, which leads to its reduction and the disappearance of its purple color (Foti, 2007; Prior et al., 2005). The efficiency of this scavenging process depends on the number and position of hydroxyl groups in the flavonoid structure. Compounds such as quercetin, with multiple OH groups, exhibit strong antioxidant capacity due to their ability to delocalize the unpaired electron and stabilize the resulting phenoxyl radicals (Heim et al., 2002).

The results presented in Table 1 reveal that the yellow variety demonstrated a higher antioxidant efficacy (EC_{50} = 0.651 ± 0.298 mg/mL) compared to the red variety (EC_{50} =

1.123 ± 0.037 mg/mL). Conflicting studies found that figs derived from dark-colored fruits (e.g., red) generally showcase a superior antioxidant capacity compared to those obtained from light-colored fruits (e.g., yellow) (Khadhraoui et al., 2019). Compared to the findings in the study by Kebal et al. (2022), the black peel fig variety had an IC_{50} greater than 0.11 mg/mL, while the red peel variety exhibited IC_{50} values below 3.11 mg/mL. In our study, the yellow variety demonstrated an IC_{50} of 0.651 ± 0.298 mg/mL, and the red variety had 1.123 ± 0.037 mg/mL, indicating stronger antioxidant activity than the red variety reported by Kebal et al. (2022). This is explained by other studies, which showed that higher levels of TPC and TFC contribute to increased DPPH free radical scavenging properties (Adli et al., 2022). In the current study, the ELE exhibits significant phenolic and flavonoid content, which correlates with potent DPPH antioxidant activity, enabling it to scavenge free radicals and reduce oxidative stress. The study parallels the findings of Nurlyana et al. (2018), who investigated the anti-oxidative properties of *F. carica* L. leaves using the DPPH assay for extracts obtained from different conditions, such as variations in extraction temperature, solvent type, and duration. Nurlyana et al. (2018) also highlighted the critical role of temperature during extraction and suggested an optimal extraction temperature of 45°C for maximizing anti-oxidative efficacy in *F. carica* L. leaf extracts. Their study also indicated a decrease in antioxidant capabilities at higher temperatures. The study by Nurlyana et al. (2018) revealed that an extraction temperature of over 50°C can degrade phenolic compounds, reducing antioxidant activity. Therefore, the current study employed a cold maceration technique at room temperature. The notable antioxidant activity of both the red and yellow extracts, as observed in our study, is attributed to their polyphenolic content as a result of the cold extraction technique. Geremu et al. (2016) highlighted the role of polyphenols in free radical scavenging due to their hydroxyl groups. These collective insights are pivotal in enhancing the understanding of the varying antioxidant potentials of different plant varieties and extraction methods.

Strengths and Limitations

This is the first study investigating two flavonoids with antioxidant properties for two varieties of *F. carica* L. cultivated in Malaysia. However, the techniques used to detect chemical composition were TLC and HPLC, which are inferior to more advanced methods, including HPTLC and LC-MS. Therefore, this is a preliminary study to provide an overview and lay the groundwork for future research using advanced methods.

Conclusion

In summary, the investigation into antioxidant properties and the chemical composition of red and yellow varieties of *F. carica* L. highlights significant insights into their phytochemical profiles and health benefits. Utilizing ethanol as the extraction solvent proved favorable, indicating its superiority in extracting diverse phytochemicals, with notable differences observed in extraction efficiency between the red and yellow varieties. Phenolic compounds and flavonoids were identified through comprehensive qualitative and quantitative analyses, which emphasize the potential health-promoting attributes of these varieties. Rutin and quercetin were identified as prominent constituents, with quercetin being predominant. Furthermore, quantification assays revealed variations in phenolic and flavonoid contents, with the yellow variety exhibiting higher phenolic levels and the red variety possessing elevated flavonoid content. Antioxidant assays affirmed the antioxidant activity of both varieties, with the yellow variety demonstrating superior efficacy. These findings underscore the importance of extraction methodologies and the significance of plant variety selection in optimizing the antioxidant potential of *F. carica* L. extracts. Overall, this research offers valuable insights for integrating these extracts into health-focused functional food products, highlighting their promising potential in promoting human health and well-being.

Abbreviations

DPPH: 2,2-Diphenyl-1-picrylhydrazyl; ELE: Ethanolic leaf extract; FRAP: Ferric reducing antioxidant power; GAE: Gallic acid equivalent; HPLC: High-performance liquid chromatography; HPLC-PDA: High-performance liquid chromatography with photodiode array detection; nm: Nanometer; PDA: Photodiode array; QE: Quercetin equivalent; TFC: Total flavonoid content; TLC: Thin layer chromatography; TPC: Total phenolic content; UV: ultraviolet.

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Supplementary Material

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