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ORIGINAL ARTICLE

Phytochemical Screening and Antioxidant Properties of *Ficus Carica* L. Leaves

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Abstract

Ficus carica L. are well known to possess a number of bioactive substances such as flavonoids and phenolic acids which have antioxidant, anti-inflammatory, and antibacterial properties. This study aimed to screen phytochemicals in the leaf and evaluate the antioxidant activities with different polarity extracts. The leaves of *Ficus carica* L. were obtained from Taman Herba in UniSZA Besut Campus, Terengganu. The leaves collected were dried under room temperature before grinded into powder. The leaf powder was then soaked in methanol at a ratio of 1:10 to obtain the crude extract and further extracted with solvent partitioning method to get hexane (HEX), dichloromethane (DCM) and methanol fractions. Total amount of phenolic and flavonoid were determined by total phenolic content (TPC) and total flavonoid content (TFC) test. Antioxidant activities of the leaves extract from different fractions were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), and ferric reducing antioxidant power assay (FRAP) assay. Thin layer chromatography (TLC) was performed to screen the chemical profile of fig leaves using mobile phase of hexane: ethyl acetate: acetic acid at ratio 7.8: 1.8: 0.4. In determination of TPC, the highest value of phenolic content obtained from MeOH crude (79.72 mg GAE/g) followed by DCM fraction (67.06 mg GAE/g), MeOH fraction (51.67 mg GAE/g) and Hexane fraction (48.27 mg GAE/g). For determination of flavonoid content, MeOH crude also possesses the highest value (77.13 mg QE/g) followed by Hexane fraction (70.33 mg QE/g), DCM fraction (52.13 mg QE/g) and MeOH fraction (31.81 mg QE/g). For all antioxidant test, MeOH fraction followed by MeOH crude obtained the highest percentage of inhibition (antioxidant activity) and possess lowest IC_{50} value as the percentage of inhibition exceed 50%. The evaluation of antioxidant properties using DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), and ferric ion-reducing assays indicates that *Ficus carica* Linn. leaves methanol crude and fraction extract possess significant antioxidant properties due to its ability to scavenge free radicals and reduce ferric ions. Screening of bioactive compounds in TLC profiling indicated presence of coumarin, flavonoids, terpenoids, saponins and phenols in the *Ficus carica* Linn. leaves extract in which methanol crude and

methanol fraction reveal a diverse array of compounds. Screening of bioactive compounds and the assessment of antioxidant activities determined the polarity strength of solvent for extraction as well as enhancing the understanding of the therapeutic potential of *Ficus carica* leaves.

Keywords: *Ficus carica L.,* fig leaves, antioxidant, bioactive compounds, different polar extracts

Introduction

The fig plant, *Ficus carica* L. is under the Moraceae family and has a long history of cultivation (Khadivi et al., 2018). Given their long history of cultivation, figs are known for their high nutritional value. Recently, the nutritional benefits of figs have led to their utilization in food preparation (Palmeira et al., 2019). The bark, roots, leaves, and fruit of fig plants, as well as its other parts, have all historically been used to treat a variety of diseases. Ficus species are particularly rich in polyphenolics and flavonoids, which are associated with antioxidant properties. Several studies have explored the plant's bioactivities, showcasing its potential in treating numerous diseases (AlShaal et al., 2019). Unfortunately, fig leaves, which possess a substantial amount of biomass and bioactive compounds, are often discarded, resulting in the wastage of resources (Alexandre et al., 2017).

Fig leaves contain various bioactive substances such as flavonoids, sugars, pectin, tannins, vitamin C, trace minerals, and others (Mustafa et al., 2021). The abundant flavonoids present in fig leaves contribute to their wide range of pharmacological effects. They have been used for treating conditions like diarrhoea, reducing blood lipids, alleviating sore throats, modulating the immune system, and preventing cardiovascular diseases. Additionally, they exhibit anti-osteoporotic properties and act as scavengers of oxidative free radicals (Pérez et al., 2015). *Ficus carica* L. leaf extracts have been reported to contain bioactive compounds such as total polyphenols, carotenoids, and flavonoids, as well as significant antioxidant properties, making them valuable as natural antioxidants.

Fig leaves were also discovered to possess a significant quantity of phenolic compounds and good antioxidant capacities (Magalhes et al., 2017). In addition, a research paper that explored the potential anti-inflammatory effects of *Ficus carica* leaf extracts demonstrated that the extracts possessed anti-inflammatory capabilities by reducing pro-inflammatory cytokine production (Sultana et al., 2018). Furthermore, the antimicrobial activity of *Ficus carica* leaf extracts against various pathogenic bacteria has significant antimicrobial effects, suggesting the potential use of fig leaf extracts as natural antimicrobial agents (Kordali et al., 2019). In a diabetic study, the leaf extract exhibited anti-hyperglycemic effects, which potentially contributing to the management of diabetes (Ali et al., 2020).

Materials and Methods

Plant material

The leaves of the fig plant were collected in Universiti Sultan Zainal Abidin (UniSZA) Kampus Tembila, located at Besut Terengganu. The fresh and healthy leaves were cleaned under running tap water to remove dirt and were further dried at room temperature. The dried leaves were ground into a powder form using a grinding mill and weighed in analytical scale.

Sample preparation

The extracts prepared by maceration method using 30 g of dried leaves powder soaked in 300 ml of methanol and placed on shaker at 120 rpm for three consecutive days before filtered through Whiteman filter paper (Abu Bakar et al., 2020). The extracts were then evaporated in a rotary evaporator to obtained the sticky paste known as methanolic crude extract.

The sticky extracts obtained were then transferred into falcon tube and kept it sealed with parafilm and the crude extracts were weighed. The falcon tube filled with crude extract was kept into a 4°C chiller. Methanol crude extracts were fractionated with methanol, dicholoromethane (DCM), and hexane solution respectively. The mixtures were left for three consecutive days and then filtered through filter paper into a new conical flask. The filtered fractions were evaporated using a rotary evaporator at temperature 40°C and kept in -20 until further use.

Thin Layer Chromatography (TLC)

The stationary phase was employed using a pre-coated TLC plates (5×10 cm). Mobile phase of hexane: ethyl acetate: acetic acid at a ratio 7.8: 1.8: 0.4 were standardized as the best mobile phase to obtain clear spot. A line was drawn on the TLC plate, 1 cm from the bottom and 0.5 cm from the top. The plate was also tested with some reagents such as iodine vapour and vanillin-sulphuric. Subsequently, the TLC plate was visualized and observed under a Spectroline UV Viewing Cabinet to be examined and identified the separated compounds (Shiny et al., 2019).

Total Phenolic Content (TPC)

Gallic acid was dissolved in a distilled water to produce a 1 mg/mL of stock gallic acid. Six distinct concentrations of the standard solution (10, 20, 30, 40, 60, 80, and 100 µg/mL) were produced. In brief, 100 µL of diluted Folin-Ciocalteu reagent (1:10) were applied after 20 µL of sample or standard. A volume of 100 µL of 7% sodium carbonate was added after the incubation period of three minutes. The mixture was re-incubated for 20 minutes at room temperature. Next, a microplate reader was used to measure the absorbance at 765 nm. The TPC was determined using the gallic acid standard curve. The data was expressed as milligrams GAE/g, or gallic acid equivalent per gramme of extract.

Total Flavonoid Content (TFC)

Six different concentrations (10, 20, 40, 60, 80, and 100 µg/mL) quercetin were prepared. In a mixture, 0.5 mL of sample extracts, 1.5 mL of 80% methanol, 0.1 mL of 1 M potassium acetate, and 0.1 mL of 10% aluminum chloride were combined, and the volume was adjusted to 5 mL with distilled water. The solution was incubated at room temperature for 30 minutes. Absorbance at 415 nm against the blank was measured using a UV/Vis spectrophotometer. The results were expressed in milligrams of quercetin equivalents per gram (mg QE/g) of the samples.

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

A stock solution of the extract was prepared at a concentration of 5 mg/mL in DMSO. As a control, a blank solution containing DMSO and DPPH were prepared. In 96-well plates, 0.1 mM of DPPH working solution were added with 200 µL of the solution added to each well containing 25 µL of different concentrations (7.8125, 15.625, 31.25, 62.5, 125, 250 and 500 mg/ml) of the sample and mixed. The mixtures were then incubated in a dark room for 30-40 minutes. Finally, the absorbance was measured at 517 nm using a microplate reader (FisherbrandTM). The DPPH scavenging activity was assessed in triplicate.

The 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)

In preparing the ABTS stock solution, an ABTS aqueous solution (7mM) were reacted with a 2.45 mM aqueous solution of potassium persulfate ($K_2S_2O_8$). The resulting mixture was kept in a dark room at room temperature for 12-16 hours to allow the reaction to occur. Subsequently, the $ABTS^+$ stock solution was adjusted with methanol until an initial absorbance value of 0.7 ± 0.02 was obtained at 738 nm. An amount of25 µL of the extracted sample were mixed with 200 µL of the diluted $ABTS^+$ solution, and the mixture was incubated for 6 minutes. Finally, the change in absorbance at 738 nm was measured using a microplate reader (FisherbrandTM).

Ferric Reducing Antioxidant Power (FRAP) Assay.

The initial solution constituting a 0.3 M acetate buffer with a pH of 3.6, was prepared by mixing 0.16 g of sodium acetate with 100 mL of 0.28 M glacial acetic acid. The pH of the solution was adjusted using 1 M HCl while for the second solution, a 0.01 M TPTZ and 0.31 g of TPTZ were mixed with 100 mL of 40 mM HCl. The third solution was prepared by diluting 0.32 g of $FeCl_3$ with 100 mL of distilled water to achieve a concentration of 0.02 M Ferric (III) Chloride. The first, second, and third solutions were combined in a ratio of 10:1:1 and heated in a water bath at 37°C for 30 minutes. Subsequently, 200 µL of the FRAP reagent were mixed with 25 µL of the sample extract, and the absorbance was immediately measured at 593 nm (Bhargavi et al., 2021).

Data analysis

The analysis were performed in triplicate and present in means with the standard deviation (SD) of three parallel measurements. Probit analysis used in determine the $IC_{50}$of extracts and fractions' antioxidant activity. Using SPSS software, the study's findings was subjected to an analysis with one-way ANOVA followed by Dunnett's technique for different comparisons with P value of 0.05.

Results

Thin Layer Chromatography (TLC)

A dark spot (Figure 1a) observed on a TLC plate when exposed to UV light at 254 nm may be due to a compound that absorbs light at this wavelength. This absorption prevents the fluorescent material in the TLC plate from receiving the UV light, leading to the appearance of a dark spot (Lisa and Nichols, 2016).

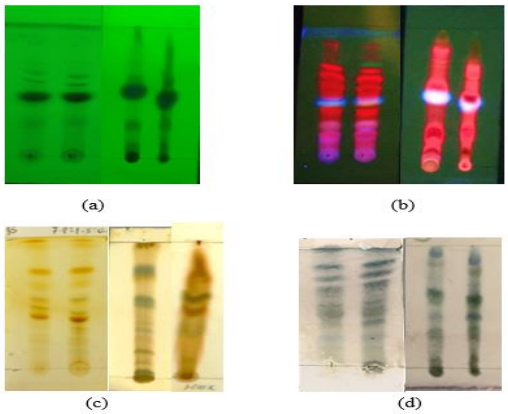

Figure 1. Detection of secondary metabolites in (a) UV 254 nm (b) UV 366 nm (c) Iodine vapor (d) Vanillin sulphuric of *F. carica* L. leaf crude and fractions

The presence of a pink color (Figure 1b) in the sample could be indicative of the existence of alkaloids or glycosides. Conversely, the occurrence of a light blue color might suggest the presence of coumarins and terpenoids. The manifestation of blue fluorescence is associated with flavonoids. The presence of red color under UV366 nm in TLC could suggest the presence of chlorophyll or steroids (Aryal et al., 2019) (Syarifah et al., 2019). Brown spots detected after exposed to iodine vapor (Figure 1c) were unsaturated compound that strongly react with aromatic compound. It was found that saturated fats lost colour more quickly than unsaturated fats, and that sugars and sugar phosphates also tended to lose colour quicker (Sims et al., 1962). The visualization with vanillin sulphuric reagent (Figure 1d), a purple colour was detected as terpenoids, dark bluish as saponins and pink colour as phenols (Masoko et al., 2012).

Total Phenolic Content (TPC)

The TPC was determined by comparing the absolute values of leaf extract solutions with standard solutions of GAE, as shown in Table 1. The TPC of the crude and fractions ranged from 48.27 mg GAE/g to 79.72 mg GAE/g. Among the three fractions, the DCM fraction had the highest phenolic content (67.06±0.04), while the hexane fraction had the lowest phenolic content (48.27±0.02). The solubility of phenols is significantly influenced by the polarity of solvents. As a result, phenols exhibit greater favorability for dissolution in polar solvents. In this context, the methanol crude extract demonstrates the highest phenolic content, followed by the DCM fraction. Conversely, the hexane fraction, being a non-polar solvent extract, contains the lowest phenolic content among the fractions.

Table 1. Total Phenolic Content (TPC) of fig leaves extracts

Solvent Extracts	TPC (mg GAE/g±SD)
MeOH Crude	79.72±0.04
Hexane Fraction	48.27±0.02
DCM Fraction	67.06±0.04
MeOH Fraction	51.67±0.01

Total Flavonoid Content (TFC)

Based on Table 2, the extracts' total flavonoid concentration ranged between 31.81 mg QE/g to 77.13 mg QE/g. The hexane fraction had the highest total flavonoid content (70.33±0.02), whereas the methanol fraction had the lowest (31.81±0.04).

Table 2. Total Flavonoid Content (TFC) of *F. carica* leaf extracts

Solvent Extracts	TFC (mg QE/g±SD)
MeOH Crude	77.13±0.03
Hexane Fraction	70.33±0.02
DCM Fraction	52.13±0.01
MeOH Fraction	31.81±0.04

Polar solvents are typically chosen to extract polyphenols from plants. Methanol is often preferred for extracting smaller polyphenol molecules due to its higher efficiency (Nakilcioglu and Otles, 2021). Therefore, flavonoids are more favor in polar solvent extracts where methanol crude shows the highest flavonoid content. However, hexane fraction which is non polar solvent contains the

highest flavonoid content among the fractions. Flavonoids are a type of secondary metabolites that are semi polar. They have a non-polar central structure that is altered with different polar hydroxyl groups and carbohydrate components, which gives them their semi-polar nature (Mehta et al., 2023). Thus, non-polar compound of flavonoid might able to be extracted in non polar solvent.

DPPH assay

The assay involves the transformation of the initially purple-colored DPPH reagent into a consistently yellow compound upon interaction with an antioxidant. The efficiency of radical composition by crude and fractions at 500 µg/mL. Methanol fraction (77.60 %) possess the highest inhibition activity followed by methanol crude (73.08 %) and DCM fraction (65.04 %) while hexane fraction (33.02 %) possess the lowest inhibition activity.

Table 3. IC_{50} and maximum inhibition concentration *F. carica* extracts and standard DPPH assay

Solvent Extracts	IC50 (µg/mL±SD)	Maximum inhibition concentration (%)
Trolox	7±0.01	83.95±0.05
MeOH Crude	82±0.04	73.08±0.07
Hexane Fraction	NA	33.02±0.11
DCM Fraction	93.4±0.06	65.04±0.06
MeOH Fraction	44±0.07	77.60±0.08

Phenolic compounds such as polar compounds, may be the potential metabolite that significantly contributes to the antioxidant activity (Swallah et al., 2020). A previous study on antioxidant potential of *F. carica* leaf found that the 70% methanolic extract of *F. carica* leaves had significant antioxidant activity with an IC_{50} of 0.06666 mg/ml (Ayoub et al., 2019). The study also revealed the presence of polyphenols, alkaloids, flavonoids, coumarins, anthocyanins, terpenoids, and saponins in the *F. carica* leaves extract. Another study reported that the DPPH radical scavenging activity of *F. carica* leaves increased from 11.31 ± 3.86% to 87.03 ± 0.15% with the addition of phenolic compounds (Abdel-Rahman et al., 2021). The study also found that *F. carica* leaves extract had anti-cancer activity.

ABTS assay

The ABTS radical cation is generation of through the reaction of ABTS salt with potent oxidizers like potassium permanganate or potassium persulfate. In this assay, antioxidants capable of scavenging ABTS are measured, resulting the intensity of the blue-green chromophore to reduce at maximum absorption at 734 nm (Ilyasov et al., 2020).

Table 4. IC_{50} and maximum inhibition concentration *F. carica* extracts and standard ABTS assay

Solvent Extracts	IC50 (µg/mL±SD)	Maximum inhibition concentration (%)
Trolox	5±0.03	93.29±0.04
MeOH Crude	194±0.07	60.94±0.03
Hexane Fraction	NA	29.87±0.15
DCM Fraction	227.74±0.08	52.45±0.05
MeOH Fraction	72.6±0.06	72.31±0.07

A previous study on ABTS free radical method found that it had significant antioxidant activity with an IC50 of 0.06666 mg/ml same as DPPH (Ayoub et al., 2019). The ABTS scavenging activity was discovered to be comparable to the DPPH activity in the current study, and a similar trend has been observed in this research. Both the ABTS and DPPH assays are based on oxidation-reduction reactions, which may account for the similarity in the results. The ABTS assay has been found to have a good correlation with the FRAP and TFC, which also rely on a single electron transfer mechanism (Rumpf et al., 2023).

FRAP assay

A method of FRAP assay was employed to measure the antioxidant capacity of various biological samples. It involves the ferric-tripyridyltriazine compound turns a vibrant blue color when exposed to low pH levels. The FRAP value is determined by comparing the change in absorbance at 593 nm in reaction mixtures with known concentrations of ferrous (Benzie et al., 1996).

Table 5. Maximum inhibition concentration of *F. carica* extracts and standard of FRAP assay

Solvent Extracts	mM (Fe2+/mg of sample±SD)
Quercetin	353.08±0.06
MeOH Crude	87.88±0.08
Hexane Fraction	75.86±0.12
DCM Fraction	82.16±0.02
MeOH Fraction	92.97±0.10

In line with the outcomes observed in the DPPH and ABTS free radical scavenging assays, the methanol fraction demonstrates robust ferric ion-reducing activities. Based on the findings of this investigation, all the extracts prove to be viable sources for the extraction of bioactive compounds with considerable antioxidant potential.

Conclusion

Throughout the chemical screening of the bioactive chemicals collected in the TLC test, methanol crude and methanol fraction display a varied array of components such as flavonoids, alkaloids, terpenoids, saponin and many other compounds. Methanol crude possesses the highest TPC and TFC. It is suggested that methanol fraction is the optimum extract for antioxidant activity as it has a high value of inhibition activity for DPPH (77.60 %) and ABTS (72.31 %) with values of IC_{50} 44 µg/mL and 72.6 µg/mL, respectively. Methanol fractions (92.97 mM Fe2+/mg) shown to be the best value to reduce ferric ions in FRAP assay.

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