Prabha Materials Science Letters
Vol. 3, No. 2, 268-282, 2024
https://doi.org/10.33889/PMSL.2024.3.2.017

Ram Arti Publishers

Solvent-Dependent Phytochemical Richness and Antioxidant Efficacy of Ficus Carica Fruits: An Experimental Study

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(Received on July 21, 2024; Revised on August 7, 2024; Accepted on August 9, 2024)

Abstract
Colorectal cancer and breast cancer is a major medical issue in Malaysia. *Ficus Carica* fruit has immense potential in the treatment of these cancers because of its antioxidant activity. This study aims to investigate the antioxidant activity of *Ficus Carica* fruit extract using various solvent extractions. The fruit extract of *Ficus Carica* was obtained using ethanolic, aqueous, and chloroform extraction methods. Preliminary phytochemical screening was conducted on ethanolic fig fruit extract. The ethanolic, aqueous, and chloroform fig fruit extracts were diluted to various concentrations and tested via antioxidant assays for total phenolic content, DPPH radical scavenging activity, and FRAP. Ethanolic extract of *Ficus Carica* fruit was observed to have the highest total phenolic content of 44.101± 0.005mg GAE/g DW, DPPH is the lowest IC50 of 0.005mg/ml and highest FRAP value of 29.423± 2.640 mg AAE/G DW at the lowest sample concentration, followed by water extraction, indicating that the highest antioxidant

activity produced by the ethanolic *Ficus Carica* fruit extract. Ethanolic extract of *Ficus Carica* fruits exhibits potent antioxidant activity.

Keywords- Ficus Carica, Antioxidant activity, Phytochemical process, Solvents.

1. Introduction

Colorectal cancer (CRC) and breast cancer (BC) are major medical concerns as they are among the leading causes of medically certified deaths (Paramasivam et al., 2022; Muhamad et al., 2023; Verma et al., 2023; NSPCRC). The literature reported that 33.9% of Malaysian females have BC especially those of Chinese ethnicity whereas 16.9% of males and 10.7% of females were diagnosed with CRC. CRC incidence rate is found to be higher in elder people (aged 65/70 and above specially from an Ethnic Chinese background) and affects them most followed by Malays and lastly Indians (Paramasivam et al., 2022). A research group noted that the development of cancer and the presence of oxidative stress are intrinsically linked. Tumor cells can modify body metabolism, leading to dysregulated glucose metabolism, which further contributes to oxidative stress (Le et al., 2019; Arfin et al., 2021; Lin et al., 2021; Talib et al., 2021; Black, 2024). The study further elaborates that the tumor cells, due to uncontrollable growth, tend to resort to glycolysis, thus generating excessive radicals. Excessive radical generation will create an oxidative microenvironment suitable for tumor growth. Excessive cellular oxidative stress in cancer is due to cancer cells promoting reactive oxygen species (ROS) and dysregulating the body's antioxidant cell defense (Kirova et al., 2022). Recent reviews claimed that the benefits of chemotherapy for patients using antioxidant supplements are that they improve chemotherapy efficacy while preserving healthy normal tissues, thus improving the patient's therapeutic outcome (Wieland et al., 2021).

Ficus Carica, or Figs, is a large genus of angiosperm plants with 850 species worldwide (AlGhalban et al., 2021). It has numerous pharmacological characteristics, including anticancer (Morovati et al., 2022), antioxidant (Shahinuzzaman et al., 2020), antiparasitic (Siyadatpanah et al., 2022), antiviral (Nirwana et al., 2018), antibacterial (Al-Snafi, 2017), antimutagen (Al-Snafi, 2017), anti-inflammatory (Liu et al., 2019), anti-angiogenic, antidiabetic (Mopuri and Islam, 2016), and antipyretic (Shamsi et al., 2020) effects. Numerous investigations have verified the presence of coumarins, triterpenoids, organic acids, phytosterols, phenolic compounds, anthocyanin content, and volatile chemicals such as aliphatic alcohols and hydrocarbons in the various plant sections of figs (*Ficus Carica)*. Figs are rich in phenolic chemicals, particularly proanthocyanidins, according to (Vinson et al., 1998). According to a different researcher, the majority of Ficus Carica cultivars frequently include volatile chemicals, organic acids, and phenolic compounds. Dried figs have been shown in another research (Khodarahmi et al., 2011) to contain benzaldehyde, hexanal and chlorogenic acid, which is essential to their antioxidant potential.

Notwithstanding the advantageous chemicals found in fig fruit, it would be good for the scientific community to conduct a thorough assessment of their antioxidant qualities utilizing various solvent extraction techniques. With the use of several solvents, the phytochemical content and antioxidant activity of fig fruits has been methodologically evaluated in this work. By elucidating the optimal extraction conditions, we hope to advance the utilization of figs in developing effective antioxidant therapies, which could potentially enhance the outcomes of conventional cancer treatments and contribute to better health management strategies. The findings are anticipated to contribute to the growing interest of evidence supporting the use of figs as a natural antioxidant source, offering potential applications in both preventive and therapeutic contexts for cancer and other oxidative stress-related diseases (Jomova et al., 2023; Marino et al., 2023; Rasool et al., 2023).

The purpose of this research is to investigate the therapeutic potential of fig fruit (Ficus Carica) in the management of breast cancer (BC) and colorectal cancer (CRC). It is imperative to identify natural substances with antioxidant capabilities that can reduce oxidative stress, given the high incidence and fatality rates of various cancers in Malaysia. By assessing Ficus Carica's phytochemical richness and antioxidant effectiveness, this study hopes to further the creation of complementary cancer therapies.

2. Methodologies

2.1 Materials

2.1.1 Source of Ficus Carica Fruits

Ficus Carica fruits were bought from Great Harvest Fruits Sdn Bhd in Batu Caves, Kuala Lumpur.

2.1.2 Chemicals Reagent

Various chemicals used in various experiments, including Mayer reagent, hydrochloric acid, Wagner reagent, Molisch reagent, concentrated Sulphuric Acid, Benedict's reagent, Glacial acetic acid, Ferric chloride solution, sodium hydroxide, Millon's reagent, Cooper Sulphate solution, Ninhydrin, Chloroform, Iodine solution, DPPH reagent, ethanol, methanol, L-ascorbic acid, gallic acid, pH 6.6 phosphate buffer, potassium ferricyanide solution, trichloroacetic acid, Folin-Ciocalteu reagent, and sodium carbonate.

2.1.3 Apparatus and Equipment

Apparatus and equipment used in this study are as follows: Bunches burner, hot plate, measuring cylinder, water bath (Memmert®), weighing machine (Mettler Toledo® B204-S), conical beaker, soxhlet apparatus set, blending machine, Micro oven, falcon test tube, vortex mixer, micropipette (Transferpette®), UV-visual spectrophotometer (Secomam® Prim).

2.1.4 Preparation and Extraction of Ficus Carica fruits

The *Ficus Carica* fruits were dried and weighed before being blended into a fine powder. The powder was then extracted with a 5:1 solvent-to-sample ratio in five separate batches. The solvent was allowed to stand for 24 hours to ensure homogenization. The liquid ethanolic was filtered and concentrated before being evaporated using a rotary evaporator. Before being used, the extract was kept at -20°C. The extract's % yield was computed with the below formula:

$$\text{Extraction yield (\%)} = \frac{Mass\ of\ extract\ (g)}{\text{Mass of dry matter (g)}} \text{ x } 100\%.$$

Five different batches of 100 g of powder were extracted using 100 ml of solvent, water, and chloroform. Under regulated temperature and lower pressure, the liquid filtrate was concentrated and allowed to evaporate. Before being used, the extract was kept in a refrigerator at -20°C (Sulaiman et al., 2015).

2.2 Phytochemical Screening of *Ficus Carica* Extract

The phytochemical screening of Ficus Carica fruits, specifically the ethanolic extract, was conducted using standard procedures to identify medicinally active substances.

2.2.1 Mayer Reagent Test for Alkaloids

1 ml of 1% hydrochloric acid and 6 drops of Wagner's reagent were added to 2 ml of extract solution. Take 2 ml extract solution. The brown reddish precipitate was produced in the presence of alkaloids.

2.2.2 Molisch Reagent Test for Carbohydrates

2 drops of Molisch's reagent were added to 2 ml of extract and shaken well followed by the addition of 2

ml of concentrated Sulphuric acid on the side of the test tube. The resulting reaction of a reddish violet ring formed reveals the presence of carbohydrates.

2.2.3 Benedict’s Reagent for Carbohydrates
The mixture of 2 ml crude extract and 2 ml of Benedict’s reagent was boiled for 10 minutes. The formation of a reddish-brown precipitate shows the presence of carbohydrates.

2.2.4 Glycosides Test
2 ml of extract solution was treated with 2 ml of glacial acetic acid mixed with 2 drops of ferric chloride solution. The formation of a brown ring at the interfaces indicates deoxy sugar characteristics.

2.2.5 Flavonoids (Alkaline Reagent Test)
2 ml of extract solution was treated with a few drops of 20% sodium hydroxide solution. The positive result for flavonoid was the appearance of an intense yellow color. If diluted hydrochloric acid is added, the yellow becomes colorless.

2.2.6 Tannins
0.5 ml of extract solution was mixed with 1 ml of distilled water and 2 drops of ferric chloride solution. The appearance of a blue color indicates the presence of gallic acids whereas green or black indicates tannins.

2.2.7 Saponins
2 ml of the extract was mixed with 20 ml of distilled water and was agitated in a vial for 15 minutes. The formation of a 1 cm layer of foam indicated the presence of Saponins.

2.2.8 Millon’s Test for Proteins
2 ml crude extract was mixed with 2 ml of Millon’s reagent. The formation of whitish precipitate which turns red upon heating gently confirms the presence of protein.

2.2.9 Ninhydrin Test for Proteins
2 ml crude extract was boiled with 2 ml of 0.2% solution of ninhydrin. The appearance of violet color reveals the presence of amino acids and proteins.

2.2.10 Steroids
2 ml crude extract was treated with 2 ml of chloroform and then the addition of concentrated Sulphuric acid sidewise. The lower chloroform layer turning reddish indicated the sample is positive for steroids. Crude extract was also treated with 2 ml of chloroform followed by 2 ml of each of concentrated Sulphuric acid and acetic acid added. The appearance of a green color indicated the presence of steroids.

2.2.11 Test for Terpenoids
2 ml crude extract was dissolved in 2 ml of chloroform and evaporated to dryness. Then mixture was treated with 2 ml of concentrated Sulphuric acid and heated for about 2 minutes. A greyish color indicates the extract contains terpenoids.

2.3 Antioxidant Screening
2.3.1 DPPH Radical-scavenging Assay for Ethanolic, Aqueous, and Chloroform Extract
The different concentrations of all plant extracts were selected as 0.005, 0.010, 0.015, and 0.02 mg/ml for

in vitro antioxidant activity. L-ascorbic acid (as a positive control) was used as the standard.

The antioxidant potential of various extracts obtained from Ficus Carica fruits was evaluated for its stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals scavenging activity based on the method outlined. Each sample solution at concentrations ranging from 0.005 to 0.02 mg/ml, was mixed with 2 ml of a 0.1 mM DPPH solution (4 mg DPPH in 100 ml methanol). After thorough mixing, the mixture was left to incubate at room temperature in darkness for 30 minutes, followed by measuring the absorbance at 517 nm using a spectrophotometer. A lower absorbance value indicated a higher capacity for scavenging free radicals.

$$\text{Radical scavenging activity (\%)} = \frac{A_C - A_S}{A_C} \times 100.$$

where, A_C is the absorbance of the control, and A_S is the absorbance of the reaction mixture containing the sample or standard.

2.3.2 Ferric (Fe 3+) Reducing Power (FRAP) Assay for Ethanolic, Aqueous and Chloroform Extracts

The reducing power assay of all extracts of *Ficus Carica* fruit was evaluated by a slightly modified method as described in the Refs (Oyaizu, 1986; Sugahara et al., 2015). Each concentration of plant extract (0.005, 0.010, 0.015, and 0.02 mg/mL) was combined with 1 mL, 0.2 M, phosphate buffer (pH 6.6) and 1 mL, 1% potassium ferricyanide [K3Fe (CN)6]. The samples then were subjected to 20 minutes of incubation at 50°C. Subsequently, trichloroacetic acid (10%) was added (1 mL) to each mixture, followed by centrifugation for 10 minutes at 1000 rpm. The resultant supernatant (1 mL) was then combined with 0.5 mL of 0.1% FeCl3 and 1 mL of distilled water. A UV-vis spectrophotometer was used to detect the absorbance at 700 nm. The standard reference was ascorbic acid, which was synthesized in a concentration range of 0.1 to 0.02 mg/ml.

Every experiment was carried out in triplicate, and the mean value ± standard deviation was used to represent the provided results. The linear regression equation, $y = mx + c$, was calculated using Microsoft Office Excel 2007. In this equation, x represents extracting concentration and y represents absorbance. The extracts' concentrations were ascertained using this regression equation. The FRAP value was then determined and represented as milligrams of ascorbic acid equivalent per gramme of extract (mg AAE equivalent) using the calculated concentrations of each extract.

$$\text{AAE} = \frac{C \times V}{M}.$$

The formula for ascorbic acid equivalent FRAP value where,
C = concentration of Ascorbic acid established from the calibration curve (mg/ml)
V = volume of extract(mL)
M = Weight of dried plant extract(g).

2.3.3 TPC Assay for Ethanolic, Aqueous, and Chloroform Extracts

Based on the techniques described in (Sharif and Bennett, 2016), the total phenolic content was calculated and reported as the gallic acid equivalent (GAE), or mg/100g of material. 10 mL of FC reagent was diluted to 100 mL with distilled water to prepare 10% Folin Ciocalteu (FC) reagent. Next, 7.5 g of sodium carbonate was diluted to 100 mL of distilled water to prepare a 7.5% sodium carbonate solution. Ethanolic, aqueous, and chloroform extracts each were prepared in concentration ranges of (0.005, 0.010, 0.015, 0.02mg/ml). 5 mL 10% Folin Ciocalteu reagent was poured into 1 mL of each extracted sample. After 5 minutes, 4 ml 7.5% sodium carbonate was added. After being left to stand for an hour at room temperature in the dark, the absorbance was measured at 750 nm using a UV-vis spectrophotometer (Ghasemzadeh etal., 2010). The experiment was repeated with Gaelic acid as standard prepared in concentrations ranging from 0.01 to 0.3 mg/ml.

$$\text{GAE} = \frac{C \times V}{M}.$$

The formula for total phenolic content where,
C = concentration of gallic acid established from the calibration curve (mg/ml)
V = volume of extract(mL)
M = Weight of dried plant extract(g).

All experiments were conducted in triplicate, and the reported data was expressed as the mean value ± standard deviation Using MS Office Excel 2007, the linear regression equation, y = mx + c, was computed, where y is absorbance, and x is extracting concentration. With this regression equation, the concentrations of the extracts were determined. Subsequently, using the calculated concentrations of each extract, the total phenolic content was calculated (Genwali et al., 2013).

3. Results and Discussion

3.1 Percentage Yield of Extracts

The percentage yield for the various solvent extracts of Ficus Carica fruits is shown in **Table 1.** The calculations are provided below:

Weight of ethanolic powder fruit extract = 100g
Final extract weight = 18.91g

$$\text{Yield \%} = \frac{Mass\ of\ extract}{Mass\ of\ dry\ matter} \times 100$$
$$= \frac{18.914}{100} \times 100$$
$$= 18.91\%.$$

The formula shown in **Table 1** is used to compute both the water extract and the chloroform extract.

Table 1. Yield percentage of Ficus Carica fruit extracts made in different solvents.

Extract	Initial weight (G)	Final weight(G)	Yield (%)
Chloroform Extract	100	2.0962	2.0962
Water Extract	100	7.0239	7.0239
Ethanolic Extract	100	18.914	18.914

According to the investigation, the ethanol extract yielded the greatest extraction yield at 18.914%. Additionally, the yields of the water and chloroform extracts were greater. The increased polarity of the solvent could be the cause of the ethanol extract's larger yield. Although the quantities of phenolic and flavonoids were lower, more polar solvents produced larger extract yields. Strong antioxidant molecules were shown to be extracted by polar solvents, as demonstrated by the polarity-dependent increase in total antioxidant activity and reducing qualities (Nawaz et al., 2020).

The study focuses on extracting fruit leaf extracts from Ficus Carica utilising different polarity solvents, such as ethanol first and chloroform second. This technique offers a broad range of extraction conditions and aids in the separation of phytochemical elements according to their polarity, which may result in a higher mass transfer of phytochemicals from plants. With increasing solvent polarity, the extraction yields rise; the ethanolic extract has the highest yield (18.9147%), while the chloroform extract has the lowest yield (2.0962). According to the study, the extraction yield of Ficus Carica fruit was 23% when 95% ethanol was used, 8.1% when water was used, and 8.1% when chloroform extraction was used, similar to another study. Ficus Carica leaves had a 4.2% chloroform extraction yield, according to the study, suggesting that the extractable parts of the fruits are mostly polar and water-soluble.

3.2 Phytochemical Screening

As shown in **Table 2**, the phytochemical screening conducted on the ethanolic extract of *Ficus Carica* fruits identified the presence of flavonoids, alkaloids, proteins, steroids, carbohydrate and terpenoids but the absence of glycosides, tannins, saponins, iodine.

Table 2. Preliminary phytochemical screening of *Ficus Carica* extract.

Secondary metabolite test & reagents		**Ethanolic extract**
Alkaloid	Mayer reagent test	+++
	Wagner reagent test	+++
Carbohydrate	Molisch reagent test	+++
	Benedict's reagent test	+++
Glycosides		---
Flavonoids	Alkaline reagent test	+++
Tannins	Ferric chloride test	+++
Saponins	Foam test	---
Proteins	Millon's test	+++
	Ninhydrin test	+++
Steroids	Liberman Buchard test	+++
Terpenoids	Conc. H_2SO_4 test	+++

#Plus sign (+) indicates positive result,
#Minus sign (-) indicates negative result.

Ficus Carica fruits contain various phytochemicals, including phytosterols, phenolic compounds, organic acids, anthocyanin, triterpenoids, coumarins, and volatile compounds, which have been found to offer numerous medicinal benefits (Kabir et al., 2017). The most common alkaloids include nicotine, cocaine, atropine, quinine, and scopolamine. Alkaloids are secondary metabolites that contain nitrogen. These organic compounds, which come from plants such as Ficus Carica fruits, Cinchona officinalis, and Atropha belladonna, have pharmacological qualities such as anti-inflammatory, anti-malarial, and anti-cholinergic actions (Debnath et al., 2018).

According to research (Rusmadi et al., 2020), the extract from Ficus Carica fruits contains 43.86% carbohydrates and 2.3-4.58% crude protein. Furthermore, antifungal proteins were discovered in the low molecular weight extract, according to a study conducted in 2023 (Bashir et al., 2023). Ficus Carica fruits contain flavonoids, which have a wide range of biological activities, such as antibacterial (Türkyilmaz et al., 2013), antioxidant (Bouaziz et al., 2015; Mahmoudi et al., 2016), anticancer, anti-inflammatory (da Gama et al., 2014), and wound healing capabilities. Anthocyanins, flavones, flavonoids, chrysin, tangerine, cinnamic acid, quince acid, aesculetin, and procyanidin are some of these constituents. Their qualities add to their health advantages.

Numerous terpenes, including limonene, menthol, α-pinene, β-pinene, linalool, and eucalyptol, were identified as possible anticancer agents in research on the volatile content of Ficus Carica (Kamran et al., 2022). It was shown that menthol and D-limonene caused apoptosis and hindered cell growth in melanoma cells, although β-caryophyllene increased the effectiveness of chemotherapy (Wróblewska-Łuczka et al., 2023).

3.3 DPPH Radical Scavenging Assay for Ethanolic, Water, and Chloroform Extract of Ficus Carica Fruits

Figure 1 shows the percentage-based free radical scavenging activity of ascorbic acid as the reference standard.

IC50 value of the standard were determined to calculate using the linear equation, y = 21038x + 35.35 with $r^2 = 0.9569$ of the standard curves of ascorbic acid where the IC50 achieved is 0.007 mg/ml.

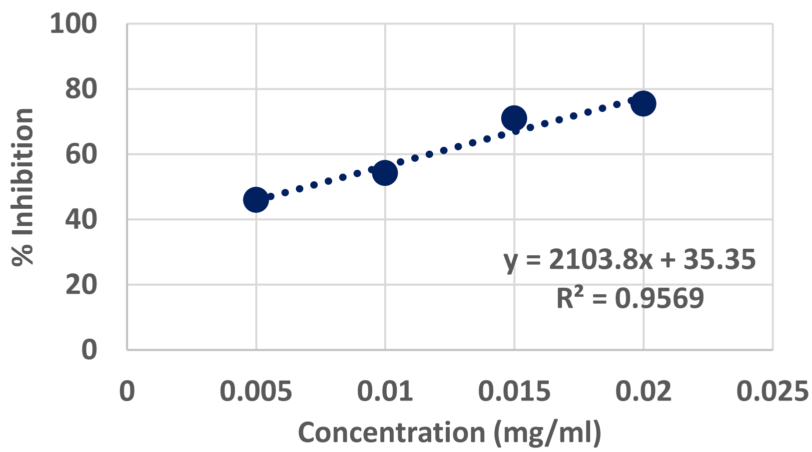

Figure 1. The percentage of free radical scavenging activity of ascorbic acid measured at various concentrations.

The DPPH free radical scavenging activity was expressed in percentage as shown in **Table 3**. An ANOVA test was performed and there was a significant comparison between all the ethanolic, water, and chloroform extracts that contain different concentrations of extracts where than 05). The values are presented as mean ± standard deviation with increasing amounts of extracted. the percentage of scavenging activity value decreases.

Table 3. The mean percentage of scavenging activity of different concentrations of ethanolic, water, and chloroform extract of *Ficus Carica* fruits.

Extract	Sample ID	Concentration of extract (mg/ml)	Percentage of scavenging % (mean ± SD)	IC50 (mg/ml)
Ethanol	**S1**	0.005	42.82 ± 0.226^{bcd}	0.005
	S2	0.01	77.95 ± 0.031^{a}	
	S3	0.015	86.39 ± 0.023^{a}	
	S4	0.02	89.75 ± 0.008^{a}	
Water	**S1**	0.005	12.75 ± 0.040^{cd}	0.014
	S2	0.01	18.41 ± 0.061^{d}	
	S3	0.015	32.71 ± 0.114^{ad}	
	S4	0.02	82.42 ± 0.017^{abc}	
Chloroform	**S1**	0.005	33.98 ± 0.101	0.023
	S2	0.01	35.66 ± 0.034	
	S3	0.015	40.88 ± 0.029	
	S4	0.02	47.25 ± 0.022	

NOTE: Means are being compared between concentration groups using post hoc turkey's test. Statistical specification was set up as P<0.05 where:
[a] Statistically significant difference with S1 containing 0.005mg of Ficus Carica extract.
[b] Statistically significant difference with S2 containing 0.01mg of Ficus Carica extract.
[c] Statistically significant difference with S3 containing 0.015mg of Ficus Carica extract.
[d] Statistically significant difference with S4 containing 0.02mg of *Ficus Carica* extract.

Table 3 shows that the IC50 of ethanolic extract, water extract, and chloroform extract of *Ficus Carica* fruit extract is (0.005 mg/ml), (0.014 mg/ml), and (0.023mg/ml) respectively. The ethanolic extract of *Ficus Carica* fruits achieved the lowest IC50 compared to other extracts as well as the ascorbic acid standard.

The DPPH test is a well-preferred method to measure the radical scavenging capability of plant extracts. It measures the drop in DPPH radical absorbance at 517 nm due to the radical's interaction with the sample's antioxidants. The primary determinant of the DPPH test is the ability to donate hydrogen to scavenge DPPH radicals (Baliyan et al., 2022). Significant antioxidants, phenolic compounds and flavonoids both destroy free radicals and have the ideal structural characteristics for scavenging them (Danet, 2021). Three extracts were employed in this study: ethanolic, aqueous, and chloroform extract, each at four different concentrations. Ascorbic acid was utilized at comparable amounts. The percentage of radical scavenging activity raised as the content of Ficus Carica extract increased, according to the data. The DPPH radical scavenging activity rises with extract concentration, following a steady trend. Ficus Carica fruits appear to have a high potential for antioxidant activity based on the IC50 values for ethanolic, aqueous, and chloroform extracts found in this study (Singh et al., 2016). A low IC50 value (less than 0.05 mg/ml) indicates significant antioxidant activity. Ficus Carica fruits may thus have a significant potential for antioxidant activity based on the IC50 values for the ethanolic, aqueous, and chloroform extracts in the current investigation, which are (0.005 mg/ml), (0.014 mg/ml), and (0.023 mg/ml), respectively.

3.4 Ferric Reducing Antioxidant Power (FRAP) Assay for Ethanolic, Water, and Chloroform Extract

Figure 2 displays the plotting of the ascorbic acid standard curve to ascertain the antioxidant capacity of the ethanolic, aqueous, and chloroform extracts. The standard curve for ascorbic acid has the equation y = 0.122x + 0.289, with an R^2 of 0.9904.

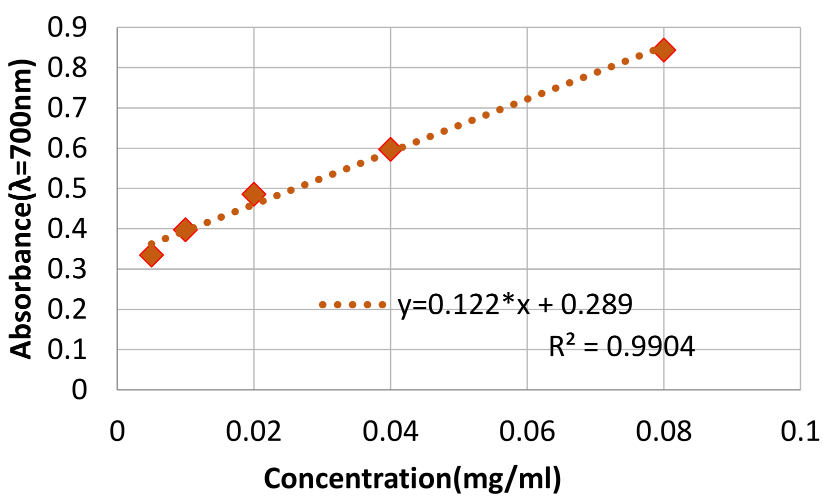

Figure 2. Standard curve of ascorbic acid.

The ferric reducing antioxidant power activity was presented in ascorbic acid equivalent, AAE as shown in **Table 4**. One way ANOVA test was carried out and there was significant difference between all sample extraction that contains difference concentration of extract where, ($P<0.05$). The values are presented as mean ± SD of triplicate samples and expressed as mg ascorbic acid equivalent (AAE) of dry weight sample.

Based on the result, the FRAP value of the ethanolic extract sample decreased with increasing concentration of extract, where lowest concentration, S1 was observed with the highest FRAP value among all concentrations, which is 29.423 ± 2.64 mg AAE/g. However, the FRAP values of water and chloroform extract samples increased with increasing concentration of extract. All concentrations of chloroform extract had lower FRAP values compared to similar concentrations of other extracts.

Table 4. Ferric reducing antioxidant power (FRAP) values of different concentration of ethanolic, water and chloroform extract of *Ficus Carica* fruits.

Extract	Sample ID	Concentration of extract, mg/ml	FRAP, mg AAE/g (mean ± SD)
Ethanolic	**S1**	0.005	29.423± 2.640^{bcd}
	S2	0.01	23.742 ± 1.412^{a}
	S3	0.015	22.831 ± 0.762^{a}
	S4	0.02	23.342 ± 1.096^{a}
Water	**S1**	0.005	-1.460± 12.421^{cd}
	S2	0.01	11.838± 3.332^{cd}
	S3	0.015	32.861 ± 2.122^{ab}
	S4	0.02	41.941 ± 2.219^{ab}
Chloroform	**S1**	0.005	-22.963 ± 3.858^{bcd}
	S2	0.01	1.642 ± 2.327^{ad}
	S3	0.015	19.6869 ± 0.471^{a}
	S4	0.02	18.772 ± 10.282^{ab}

NOTE: Means are being compared between concentration group using post hoc turkey's test. Statistical significance was set up $P<0.05$ where:
[a] Statistically significant difference with S1 containing 0.005mg of Ficus Carica of ethanolic extract.
[b] Statistically significant difference with S2 containing 0.010mg of Ficus Carica of ethanolic extract.
[c] Statistically significant difference with S3 containing 0.015mg of Ficus Carica of ethanolic extract.
[d] Statistically significant difference with S4 containing 0.02mg of Ficus Carica of ethanolic extract.

Reducing power is a crucial indicator of antioxidant activity, as it indicates the ability of compounds to diminish oxidized intermediates in lipid peroxidation processes(Manach et al., 2004.). In this study, the ethanol extract of *Ficus Carica* fruits showed promise for antioxidant phytoconstituents, as it demonstrated a higher reduction capability compared to conventional ascorbic acid (Hue et al., 2020). The increasing trend of FRAP value of all extracts except the ethanolic extract of fig fruits when sample concentration increased was in line with previous studies, suggesting antioxidant activity increases with sample concentration (Sukandar et al., 2017). Nevertheless, prior research yielded no explanation for the declining pattern of the ethanolic extract's FRAP value as sample concentration rose, which may have been caused by an inadequate reagent-to-sample ratio.

The ethanolic fig extract had the greatest FRAP value at the lowest concentration, followed by the aqueous extract and the chloroform extract. The ethanolic extract of Annona muricata seeds had comparable outcomes, with the highest FRAP value being 369.84+7.96 mg AAE/100g DW. The subsequent highest result was chloroform extract with 129.94+14.02 mg AAE/100g DW, and the lowest result was aqueous extract with 93.91+9.71 mg AAE/100g DW. The FRAP results were consistent with the total phenolic content of muricata seeds extract, decreasing in the same extract sequence: ethanolic > chloroform > aqueous.

3.5 Total Phenolic Content (TPC)

3.5.1 TPC For Ethanolic Extract, Water, and Chloroform of *Ficus Carica* Fruit Extract

The standard curve for gallic acid was plotted as shown in **Figure 3**. to determine the concentration of total phenolic contents of the ethanolic, water, and chloroform extract. The equation of the ascorbic acid standard curve was $y = 0.64x + 0.188$ with $R^2 = 0.991$.

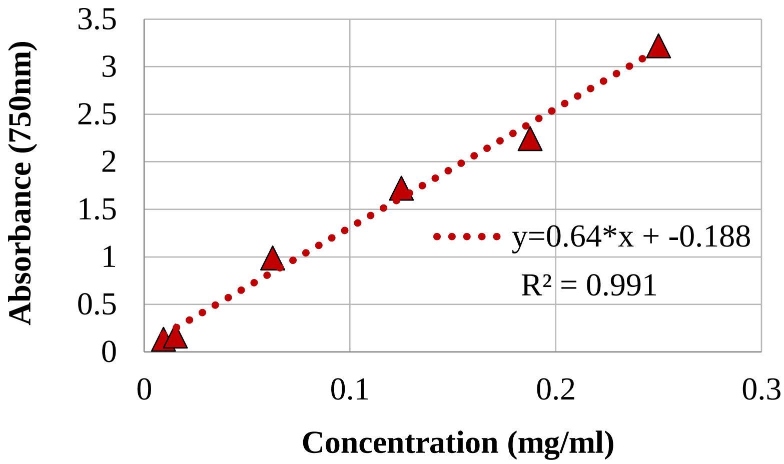

Figure 3. Standard curve of gallic acid.

The total phenolic content was expressed as gallic acid equivalent, GAE as shown in Table 5. One-way ANOVA test was carried out and there was a significant difference between all sample extractions that contained a different concentration of extract where ($P<0.05$). The samples were experimented in triplicate and are presented as mean ± SD, expressed as mg gallic acid equivalent (GAE) of dry weight sample (mg GAE/g DW). According to the result, the phenolic content of ethanolic extract is not significantly different among different concentrations. However, the phenolic content of water and chloroform extract samples increased with the rising concentration of extract. At the lowest concentration S1, the ethanolic extract had the highest phenolic content among extracts with a value of 44.101± 0.005 mg GAE/g.

Table 5. Mean total phenolic concentration of different concentrations of ethanolic *Ficus Farica* fruit extract.

Extract	Sample ID	Concentration of extract mg/ml	Mean of TPC mg GAE/g (mean ± SD)
Ethanolic	**S1**	0.0625	44.101± 0.005
	S2	0.125	41.089 ± 0.012
	S3	0.25	27.842 ± 0.033
	S4	0.5	26.032 ± 0.010
Water	**S1**	0.0625	22.947± 0.007^{bcd}
	S2	0.125	25.4352 ± 0.001^{abc}
	S3	0.25	26.362 ± 0.015^{ab}
	S4	0.5	25.7148 ± 0.066^{ab}
Chloroform	**S1**	0.0625	-24.861± 0.060^{bcd}
	S2	0.125	10.2024 ± 0.045^{a}
	S3	0.25	19.698 ± 0.010^{a}
	S4	0.5	22.54 ± 0.070^{a}

NOTE: Means are being compared between concentration groups using post hoc turkey's test. Not statistically specification was setup $P<0.05$ where,
a Statistically significant difference with S1 containing 0.0625mg of Ficus Carica extract.
b Statistically significant difference with S2 containing 0.125mg of Ficus Carica extract.
c Statistically significant difference with S3 containing 0.25mg of Ficus Carica extract.
d Statistically significant difference with S4 containing 0.5mg of Ficus Carica extract.

Phenolic compounds are metabolizers produced by plants' secondary pathways, (Luna-Guevara et al., 2018) and their antioxidant activity is not linked to total phenolic concentration (Yang et al., 2002). This study found no relationship between antioxidant activity and total phenolic content in *Ficus Carica* fruits, despite several research showing a link between phenolic concentration and antioxidant capability. The absence of a relationship between total phenolic content and antioxidant ability in plant samples can be attributed to the inclusion of phytochemicals carbic acid, tocopherol, and pigments, as well as their synergistic interactions (Bajpai et al., 2005).

The total phenolic content of all fig extracts except ethanolic extract displayed a concentration-dependent increase(Sengul et al., 2009), as observed in previous studies. However, a descending trend of total phenolic content was observed for ethanolic fig extract, suggesting that the reduction of phenols in the sample was not completely exhausted due to insufficient Folin-Ciocalteu reagent-to-sample ratio at higher concentrations (Molole et al., 2022). Among all constituents, phenolic compounds are predominantly important in plants as free radical scavengers, metal-chelating agents, and chain-breaking antioxidants. The varying TPC values of Ficus Carica extracts suggested that the fruits contain phenolics of varying polarities. Ficus Carica fruits are mostly composed of polar phenolic compounds, while certain semi-polar and non-polar phenolic compounds were also found in significant amounts.

4. Conclusion

The ethanolic, aqueous, and chloroform extracts of Ficus Carica fruits exhibited significant antioxidant activity, which is attributed to the presence of phenolic compounds in the fruit. Notably, the ethanolic extract demonstrated superior antioxidant activity, indicating a higher concentration of bioactive compounds with potential therapeutic benefits for cancer treatment. Based on these findings, we recommend future in vitro studies to investigate the anticancer activity of the ethanolic extract of Ficus Carica fruits using MCF-7 breast cancer and CaCO-2 colon cancer cell lines. This study will further substantiate the therapeutic potential of this extract in cancer treatment.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

The authors gratefully thank the University of Cyberjaya for funding their study (Grant No. URGS/03/07/2022).

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