Phytochemical Screening of Bioactive Compounds and Antioxidant Activity of Different Extracts from the Fruits and Barks of *Ficus racemosa*

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Abstract

Ficus racemosa, commonly known as the fig tree, is recognized for its broad spectrum of medicinal properties. Its potential as a source of natural antioxidants has attracted growing scientific interest. This study aimed to identify phytochemical compounds and evaluate the antioxidant activity of fruit and bark extracts of *Ficus racemosa* using solvents of increasing polarity—methanol, ethyl acetate, and hexane. Dried plant materials were extracted successively at room temperature through cold maceration. Phytochemical screening was conducted to detect major secondary metabolites, and antioxidant activity was assessed using the DPPH free radical scavenging assay. The screening confirmed the presence of alkaloids, flavonoids, and tannins in both fruit and bark extracts. Methanol and ethyl acetate fruit extracts demonstrated strong antioxidant activity with IC_{50} values of 67.114 µg/mL and 69.149 µg/mL, respectively. Meanwhile, methanol and hexane bark extracts exhibited moderate antioxidant effects with IC_{50} values of 123.043 µg/mL and 124.137 µg/mL. Quercetin, used as the standard, showed very strong antioxidant activity with an IC_{50} of 4.975 µg/mL. These findings suggest that *Ficus racemosa*, particularly its fruit extracts, has promising antioxidant potential and may serve as a valuable natural agent in preventing free radicals.

Keywords: *antioxidant, bioactive compounds, DPPH, Ficus racemosa, phytochemical screening*

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Highlights

1. Ficus racemosa fruit and bark contain alkaloids, flavonoids, and tannins.
2. Sequential extraction used methanol, ethyl acetate, and hexane solvents.
3. Fruit extracts exhibited strong antioxidant activity (IC50 < 70 µg/mL).
4. Bark extracts showed moderate antioxidant potential compared to fruit.
5. Ficus racemosa fruit extracts are promising as natural antioxidant sources.

Introduction

For centuries, plants have given huge, promising benefits as a natural medicinal compound (Okselni et al., 2024). Current research emphasizes the rational use of medicinal plants to ensure long-term availability, notably in Chinese herbal medicine (Xiang et al., 2025). A part of the leaves, fruits, flowers, barks, stems, latex, and roots of plants are plentiful and have a wide range of phytochemical constituents with unique characteristics (Tikent et al., 2025; Wu et al., 2025). Chemical constituents, especially the secondary metabolites of plants, mainly influence their biological activity (Okselni et al., 2024). The extracts contained various classes of phytochemicals such as alkaloids, flavonoids, phenols, and steroids/terpenoids. Notably, flavonoids are a subclass of phenolic compounds, both of which are well known for their diverse pharmacological properties (Elsherif et al., 2023).

Ficus racemosa (*F. racemosa*) belongs to the genus *Ficus* in the family Moraceae, typically referred to as ara or elo in Indonesia. These plants predominantly thrive in tropical and subtropical regions of Australia and Asia. Some reports based on modern research have confirmed that distinct segments of *F. racemosa* exhibit traditional wound healing (Bopage et al., 2018), (Katkar et al., 2024), anti-diarrhea (Bheemachari et al., 2007), antioxidant (Veerapur et al., 2011), anticancer (Sivakumar et al., 2019), antidiabetic (Ahmed et al., 2011; Ravichandiran et al., 2012), antibacterial (Gardia et al., 2021), and anti-inflammatory (B.N. et al., 2021). Both natural and synthetic antioxidants have been widely applied in medicinal practice to address oxidative stress triggered by free radicals. Secondary metabolites of *F. racemosa* were alkaloids, tannins, saponins, lupeol (Chaware et al., 2020) (Kusuma Dewi & Suryani, 2024; Suryani & Gustiana, 2023), and flavonoids (Hidayanti et al., 2023). Studies have shown that the methanolic extract of *F. racemosa* fruits was reported to have scavenging activity against DPPH radicals with an IC_{50} value of 65.042 µg/mL (Hidayanti et al., 2023). The ethanolic extract of *F. racemosa* fruits showed a DPPH radical scavenging EC_{50} value of 28.4 114± 0.50 µg/mL (Tamuly et al., 2015).

However, profiling chemical screening with the antioxidant activity of different extracts with a gradual solvent extraction from *F. racemosa* has not been reported. This research reported the main objectives of the phytochemical screening of bioactive compounds and antioxidant activity against DPPH radicals of different extracts with a gradual solvent extraction of *F. racemosa.*

Research Methods

Materials

2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin, HCl, Mg powder, methanol, ethyl acetate, hexane, Dragendorff's reagent, ferric chloride ($FeCl_3$), and Liebermann Burchard's reagent were purchased from Merck and Sigma-Aldrich. The chemicals used in the analysis were of analytical grade.

Instrumentation

The tools utilized in this research are an oven (Memmert), an electronic balance (Kern), a hotplate (Thermo Scientific), a rotary evaporator (IKA, RV 10 digital), a UV-VIS spectrophotometer (Shimadzu UV-1900i), an electric grinder (Q2-8050), and a micropipette (Eppendorf).

Procedure

1) Plant materials and sample extraction

   As evidenced by the research, fruit and bark samples were collected from different populations. Fruit samples were collected from Janapria Village, Central Lombok (8.7014° S, 116.3899° E), while bark samples were collected from East Bagik

Payung Village, East Lombok (8.6281° S, 116.5669° E). The botanical identity of the specimens was verified as *Ficus racemosa* through consultation with taxonomic literature and confirmed via an online database (Royal Botanic Gardens, 2023). Subsequently, both samples were cleaned under running water and dried in an oven at 60 °C for about 6 hours. Each dried sample was pulverized using a 150-W electric grinder and passed through a 60-mesh sieve.

To obtain a different extract, methanol extract of fruits (MEF), ethyl acetate (EtOAc) extract of fruits (EEF), hexane extract of fruits (HEF), methanol extract of barks (MEB), ethyl acetate extract of barks (EEB), hexane extract of barks (HEB), each dried sample (2.5 g) was accurately weighed and extracted gradually from hexane (non-polar), ethyl acetate (semi-polar), and methanol (polar solvent). The extraction process was carried out at room temperature for 24 hours using 250 mL of solvents with varying polarities, and each sample was processed in triplicate. After extraction, the solutions were filtered and concentrated by evaporation. The resulting crude extracts were then stored in a refrigerator until further analysis.

2) Phytochemical assay

Phytochemical screening of the extracts of *F. racemosa* was tested using a qualitative slight modification method as previously described (Ouandaogo et al., 2023), (Jabeen et al., 2023), (Prajapati et al., 2024).

3) Test for flavonoid (Shinoda test

Each extract (5 mL) was placed into a tube, and 2 g of Mg powder was added to each test tube. A flavonoid was present when a few drops of HCl were dispensed, and the mixture formed a yellow to magenta color.

4) Test for alkaloids (Dragendorff's test)

A volume of each extract (2 mL) was diluted with a 1:9 ratio of HCl-distilled water. Alkaloids are confirmed by the visible change to reddish-brown precipitate when five drops of Dragendorff reagent are added to the mixture test tube.

5) Test for tannins (ferric chloride test)

2 mL of extract was treated with 3 mL of a 5% $FeCl_3$ solution. The presence of tannins implies a bluish-black or greenish-brown color.

6) Test for saponins (Froth test)

Each extract with a ratio of 1:5 was diluted in distilled water and vigorously shaken for 20 s. The presence of saponins showed a stable foam after 30 min.

7) Test for steroid/triterpenoids (Liebermann Burchard's test)

Five drops of Liebermann-Burchard reagent were added to 2 mL of each extract. A layer with a green or blue ring on top of the surface of each extract indicates the presence of steroid/triterpenoid. In the table results, denoted by (+) suggests the presence and (-) the absence of phytochemical secondary metabolite.

8) Radical scavenging assay

The radical scavenging assay (RSA) of each extract was determined by reducing the DPPH, as performed and adapted by former research (Hidayanti et al., 2023; Liu et al., 2024).

9) Preparation of quercetin and test solution

A variety of concentrations of MEF, EEF, and HEF were prepared at 25, 50, 75, 100, and 125 ppm. Similarly, MEB, EEB, and HEB were prepared at concentrations of 50, 100, 150, 200, and 250 ppm. To construct the calibration curve, a stock solution of quercetin (100 ppm) was prepared by dissolving 2.5 mg of quercetin in 25 mL of methanol. The quercetin was then diluted to obtain working

concentrations of 2, 4, 6, 8, and 10 ppm.

10) DPPH assay

Each concentration of quercetin and the test solution was mixed with the DPPH solution. The test solution (1 mL), methanol solvent (1 mL), and the DPPH (2 mL) were mixed and incubated at room temperature for 30 min in the absence of light, followed by measurement of absorbance at 516 nm using a UV-VIS Spectrophotometer with three replicates. The radical scavenging activity was calculated following Equation 1 (Krüzselyi et al., 2023). The basis for calculating the IC_{50} value, as expressed as the half maximum inhibitory concentration of each extract, was the calibration curves (Srinivasan & Lloyd, 2024), where A_b is the absorbance of the blank (DPPH solution without sample), and $A_{s,}$ is the absorbance of the mixture of DPPH with each extract or quercetin.

$$\text{RSA (\%)} = \left(\frac{A_b - A_s}{A_b}\right) \times 100\% \qquad (1)$$

Results and Discussion

Qualitative phytochemical analysis

Phytochemical screening was conducted to recognize and explore potential bioactive compounds responsible for the plant's pharmacological activities (Itam et al., 2021). The summary of the phytochemicals of each extract is shown in Table 1.

Table 1. *F. racemosa* fruits and bark extract qualitative profile

Secondary metabolite	Extracts					
	MEF	**EEF**	**HEF**	**MEB**	**EEB**	**HEB**
Flavonoids	+	+	-	-	-	-
Alkaloids	-	-	-	-	-	+
Tannins	+	+	-	+	-	-
Saponins	+	-	-	-	-	-
Steroid/triterpenoid	+	+	+	-	-	-

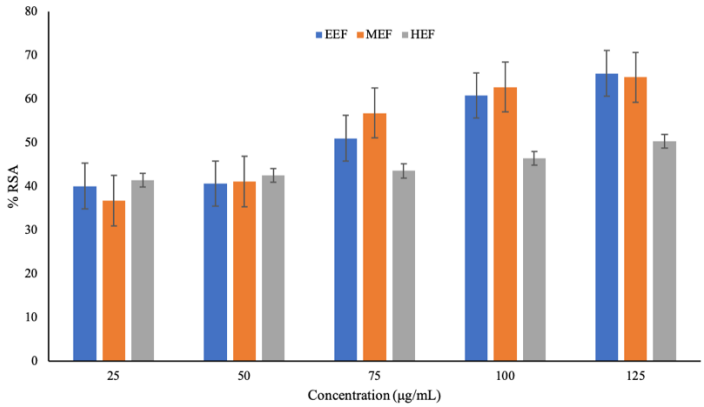
Phytochemical analysis of the MEF indicated the presence of steroids, tannins, saponins, and flavonoids; then the EEF presented flavonoids, tannins, and steroid/triterpenoid, whereas alkaloids and saponins were absent. As previously acknowledged, the methanolic extract of the fruit also contains flavonoids, steroids, tannins, and saponins (Hidayanti et al., 2023). Furthermore, qualitative testing of the aqueous infusion of *F. racemosa* fruit confirmed the presence of both flavonoids and tannins (Suryani et al., 2025), supporting the notion that these bioactive constituents are widely distributed across different extract types. The HEF primarily contains steroids/triterpenoids. The MEB is known to include tannins, and the HEB only contains alkaloids, whereas the EEB yielded no positive findings for any bioactive substances. These findings are consistent with previously reported (Chaware et al., 2020; Shi et al., 2018). Conversely, the plants are the same as those previously studied; their chemical bioactive profiles may differ due to variation in environmental conditions that influence local ecological interactions (Ismail et al., 2017; Hasana et al., 2024), analytical variations, and organ-specific accumulation of plant (Lv & Guo, 2023; Y. S. Shi et al., 2022).

The phytochemical screening of *F. racemosa* fruit and bark extracts, prepared using solvents of increasing polarity, hexane, ethyl acetate, and methanol, demonstrated a diverse range of bioactive constituents distributed

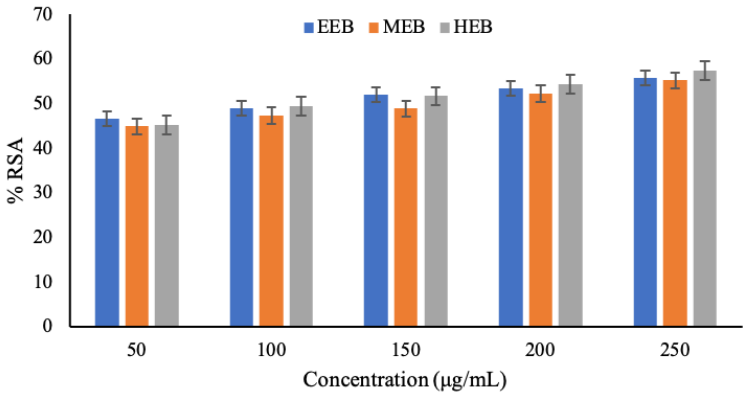
according to solvent polarity. Non-polar extract with hexane was rich in lipophilic compounds such as triterpenoids. At the same time, more polar solvents, ethyl acetate and methanol, were more effective in extracting alkaloids and phenolic compounds, including flavonoids and tannins (Rachma Novitasari et al., 2023). Irrespective of theoretical assumptions, an ideal extraction method should be low-cost, scalable, and easy to implement. Hence, the refinement of classical maceration using gradual solvents from non-polar to semi-polar and then to polar solvents represents an important development aimed at optimizing the recovery of bioactive compounds. In line with earlier studies, the polarity principles of the solvent assumed in extraction additionally affect the bioactive compounds that can be extracted. In general, stepwise cold maceration involves the sequential use of solvents with increasing polarity, starting with non-polar solvents, followed by semi-polar solvents, and finally polar solvents (Azwanida, 2015).

Antioxidant activity

It is widely recognized that antioxidant capacity analysis was employed in the DPPH assay. As a stable radical, DPPH is recognized due to the delocalization of its unpaired electron across the entire molecule (Meray et al., 2024). The data demonstrated that based on Figure 1, all extracts of *F. racemosa* have different RSA percentages, and this causes different antioxidant abilities. The RSA percentages reflect the ability of the extract at a given concentration to inhibit 50% of DPPH radicals. A higher RSA percentage at a lower concentration indicated a stronger antioxidant potential of the extract. This is supported by the IC_{50} value, where a lower IC_{50} corresponds to a higher antioxidant capacity (Olszowy-Tomczyk, 2021).



(a)



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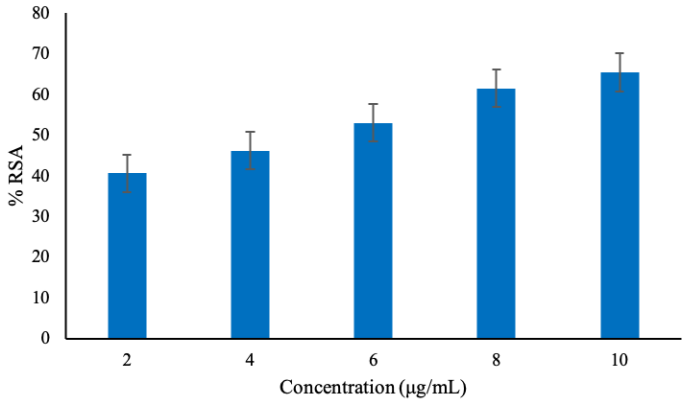

(c)

Figure 1. Comparison of the % RSA vs concentrations of (a) the fruit extract, (b) bark extract, and (c) quercetin

In this study, the antioxidant activity of fruit and bark extracts, obtained using methanol, ethyl acetate, and hexane, was assessed based on their % RSA across a range of concentrations. The % RSA values, which reflect the ability of each extract to neutralize free radicals, increased proportionally with concentration, indicating a clear dose-response relationship. Among the fruit extracts, MEF and EEF displayed notably higher % RSA values compared to HEF, suggesting that polar solvents are more efficient in extracting antioxidant-active compounds. These findings are in line with previous research, which reported % RSA values increase from 54.409 % to 61.783% with the following concentration: 75 ppm to 125 ppm (Hidayanti et al., 2023). Another finding, *F. racemosa* with aqueous solvents reported the highest % RSA range of 59.21 % to 90.94 % at concentrations of 125 ppm to 500 ppm (Suryani et al., 2025). The differences in activity may be attributed to the varying content of bioactive compounds in each extract (Flieger et al., 2021). To evaluate the strength of this relationship, linear regression analysis was performed. All extracts exhibited strong positive correlations between concentration and % RSA, with R^2 values ranging from 0.912 to 0.989. Specifically, MEF, EEF, and HEF yielded R^2 values of 0.929, 0.951, and 0.912, respectively, while MEB, EEB, and HEB demonstrated slightly higher R^2 values of 0.989, 0.986, and 0.989. All data are shown in Table 2.

Table 2. Linear regression extract and quercetin

Extract	Linear Regression Equation
MEF	$y = 0.3126x + 29.02$ ($R^2 = 0.929$)
EEF	$y = 0.2869x + 30.161$ ($R^2 = 0.951$)
HEF	$y = 0.0864x + 38.353$ ($R^2 = 0.912$)
MEB	$y = 0.046x + 44.34$ ($R^2 = 0.989$)
EEB	$y = 0.051x + 41.99$ ($R^2 = 0.986$)
HEB	$y = 0.058x + 42.80$ ($R^2 = 0.989$)
Quercetin	$y = 3.25x + 33.83$ ($R^2 = 0.989$)

These results confirm that the selected concentration ranges were appropriate to capture the full dose-response behaviour of each sample. In addition to R^2, the slope of the regression line provided further insight into the efficiency of each extract per unit concentration (Rohmah et al., 2020). The MEF and EEF showed

steeper slopes (0.3126 and 0.2869), indicating greater radical scavenging efficiency, whereas HEF had a lower slope (0.0864), reflecting weaker activity. The bark extracts had smaller but consistent slopes (ranging from 0.046 to 0.058), suggesting lower antioxidant potency per concentration unit. The concentration ranges for both the plant extracts and the quercetin standard were deliberately selected based on their antioxidant potential. Broader concentration intervals were used for the extracts to ensure sufficient detection of activity across the dose-response curve. In contrast, quercetin, known for its high antioxidant activity (Vo et al., 2019), was tested at lower and narrower concentrations to avoid saturation effects and to allow for accurate modelling.

In the extraction of plant-derived bioactive compounds, the selection of solvents is guided by the principle of "like dissolves like". This principle implies that compounds with physicochemical properties similar to those of a given solvent are more likely to be efficiently extracted, whereas compounds with dissimilar properties exhibit limited solubility and extraction efficiency (Peiris et al., 2023). Phenolic compounds, including flavonoids as one of their major subclasses, are known for their strong antioxidant properties and typically exhibit polar characteristics. As such, they are more efficiently extracted using polar or semi-polar solvents. Conversely, non-polar solvents are more suitable for isolating non-polar constituents from plant materials (Flieger et al., 2021).

The MEF was macerated with a polar solvent, specifically methanol, and then the EEF was macerated with a semi-polar solvent, ethyl acetate, allowing for the extraction of bioactive chemicals that range from semi-polar to polar. Plants are rich sources of phenolic compounds, including flavonoids, which are generally soluble in semi-polar to polar solvents, making these solvents suitable for their efficient extraction (Abdel-Rahman et al., 2021). Unlike the HEF, which is macerated with a non-polar solvent like hexane, it is easier to extract non-polar components such as lipids and terpenoids. Thus, based on the % RSA of fruit extract, it is clear that the MEF and the EEF are more effective than the HEF in reducing DPPH activity. The % RSA of the MEF and the EEF at 75 µg/mL was able to inhibit 56.787% and 51.004% of DPPH activity; however, the HEF could only block less than 50%, specifically 43.534%. At the highest concentration of 125 µg/mL, the MEF and the EEF inhibited DPPH activity by 64.980% and 65.863%, while the HEF inhibited DPPH activity by 50.281% specifically. This suggests that the bioactive chemicals capable of suppressing DPPH activity are more abundant in the MEF and the EEF than in the HEF. The application of a solvent's polarity gradient influences the antioxidant capacity classification differently for each extract.

The percentage of RSA in the bark extracts of *F. racemosa* is more diverse. The bark extracts inhibited DPPH radical by 50% at a concentration of 150 µg/mL, with the MEB inhibiting radicals by 51.971% and the HEB inhibiting by 51.619%, respectively. Compared to the EEB, which has not yet been shown to impede up to 50% at a concentration of 150 µg/mL. When the concentration was raised to 200 µg/mL, the EEB could only suppress DPPH radicals by 52.147%. This data phenomenon differs from the fruit extract results, which demonstrate that the % RSA with polar and semi-polar solvents is significantly larger than in non-polar. Nevertheless, the % RSA of the bark extract obtained in non-polar, namely hexane, is somewhat greater than other solvents evaluated at the same concentration. This is possible because the diversity of active chemical compounds in a plant varies between its parts. In contrast to the data in Figure 1, quercetin's capacity to inhibit DPPH

radicals at a concentration of 6 µg/mL has increased to 52.993%. The studies reveal that quercetin has a high antioxidant capability.

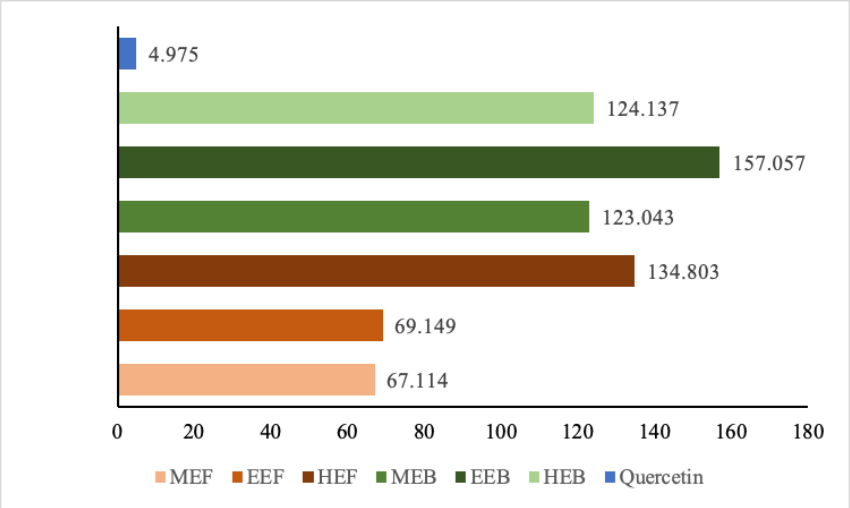

Figure 2. IC_{50} (µg/mL) of extract and quercetin

As shown in Figure 2, the MEF exhibited the lowest IC_{50} value among all tested plant extracts, excluding quercetin, which served as a standard reference. This result aligns with previous findings, in which the IC_{50} values of the MEF were reported at 65.042 µg/mL (Hidayanti et al., 2023), indicating strong antioxidant activity. According to another study, IC_{50} values within the range of 50-100 µg/mL are considered indicative of high antioxidant potential. The phytochemical content of the MEF reported in Table 1 includes flavonoids, tannins, saponins, and steroids/triterpenoids, further supporting the observed activity, as these compounds are known contributors to radical scavenging mechanisms (Mustarichie et al., 2017).

The HEB, which is positive for non-polar alkaloids, exhibited a lower IC_{50} value than the EEB. This finding is consistent with previous studies on non-pungent pepper cultivars, where hexane extract showed superior DPPH RSA with an IC_{50} value as low as 0.48 µg/mL, outperforming extracts obtained using more polar solvents (Bae et al., 2012). Similarly, research on *Portulaca oleracea* revealed that hexane extracts had strong superoxide radical activities, with an IC_{50} value of 14.36 ± 2.17 µg/mL (Chen et al., 2022). In the same context, the chloroform extract of *F. carica* fruit exhibited considerable antioxidant capacity, with an IC_{50} value of 0.023 mg/mL (equivalent to 23 µg/mL) (Yeasmin et al., 2024). These findings indicate that hexane is particularly successful at extracting non-polar antioxidant molecules, which have powerful antioxidant properties. Fortunately, it is crucial to highlight that the total antioxidant activity of plant extract is the consequence of a complex interaction of numerous components. While hexane is excellent at extracting non-polar antioxidants, ethyl acetate and methanol, with their mild and polar properties, are more effective at extracting polar chemicals such as phenolics, including flavonoids, which are recognized for their antioxidant effects (Rao et al., 2023).

Conforming to Figure 2, the IC_{50} value of quercetin, measured at 4.975 µg/mL, indicates a very powerful antioxidant capacity, as it falls within the highly active category, $IC_{50} < 50$ µg/mL (Mustarichie et al., 2017). This potent activity is closely linked to the molecular structure of quercetin, particularly the

presence of multiple hydroxyl (OH) groups, which exhibit stronger DPPH radical scavenging effects, highlighting the role of hydroxylation in the antioxidant mechanism (Hosoya et al., 2024). Thus, polyphenol compounds that have a higher number of OH groups are likely to inhibit free radicals by donating a proton to stabilize them (Vo et al., 2019). As a bioflavonoid, quercetin has been widely reported to modulate oxidative stress through its radical-scavenging properties and by influencing the activity of endogenous antioxidant enzymes. Supporting this, Alharbi's research found that dietary supplementation with quercetin significantly enhances the body's antioxidant defense system (Alharbi et al., 2025).

Conclusions

This study highlights that extracts of *F. racemosa*, obtained through sequential solvent extraction, possess significant antioxidant activity, particularly those derived using the methanol extract of fruit (MEF) and ethyl acetate extract of fruit (EEF). These findings emphasize the importance of solvent polarity in optimizing the extraction of phytochemicals with antioxidant potential. The strong and moderate antioxidant activities observed suggest that *Ficus racemosa*, especially its fruit extracts, may serve as a promising source of natural antioxidants. Future investigations are recommended to isolate and elucidate the chemical structures of the active constituents responsible for the observed bioactivity in MEF and EEF extracts.

Author Contributions

NS designed the research study. **YD**, **NS**, and **BRH** performed the research. **YKD** and **BAAM** provided help and advice on analyzing and visualizing the data. **NS**, **YKD**, and **BAAM** wrote the manuscript. All authors contributed and agreed to the final version of the manuscript.

Conflict of Interest

The authors have declared that there is no conflict of interest.

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