Antioxidant Activity of Weeping Fig (Ficus benjamina L.) Leaf Extract: A Comparative Analysis Using ABTS and FRAP Assays

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ABSTRACT

Ficus benjamina L. (weeping fig) has been traditionally used in folk medicine, but its antioxidant potential remains scientifically underexplored. This study aimed to comprehensively evaluate the in vitro antioxidant activity of a 70% ethanolic extract of F. benjamina leaves using two assays. The extract was prepared by maceration and subjected to phytochemical screening. Antioxidant activity was assessed through the ABTS radical scavenging assay, reported as IC_{50} and Trolox equivalents (TE), and the FRAP reducing power assay, reported as $FeSO_4$ equivalents (FE). Phytochemical analysis revealed abundant flavonoids, phenolics, and tannins. The extract demonstrated potent antioxidant activity, with an IC_{50} of 28.4 µg/mL in the ABTS assay and a value of 412.3 mg TE/g. The FRAP assay confirmed strong reducing power, with a value of 1850.6 µmol FE/g. A very strong positive correlation (r = 0.978) was found between the results of the two assays. While less potent than pure ascorbic acid, the extract's activity is significant for a plant extract. The 70% ethanolic extract of F. benjamina leaves possesses substantial in vitro antioxidant activity, attributed to its high phenolic and flavonoid content. The strong correlation between ABTS and FRAP results indicates complementary antioxidant mechanisms.

KEYWORDS

Ficus benjamina, antioxidant, ABTS assay, FRAP assay, Phenolic Compounds, Natural Exctract

INTRODUCTION

The increasing prevalence of oxidative stress, induced by an imbalance between free radicals and the body's antioxidant defense system, is a fundamental contributor to the

pathogenesis of numerous chronic diseases, including cancer, cardiovascular disorders, neurodegenerative conditions, and diabetes (Liguori et al., 2018). Synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been widely used to combat oxidative damage. However, their use is now being scrutinized due to potential health risks and consumer preference for natural alternatives (Carocho et al., 2018). This has propelled scientific exploration towards discovering safe and effective antioxidants from botanical sources.

Plants are a rich reservoir of bioactive compounds, particularly phenolics, flavonoids, and tannins, which are renowned for their potent free radical-scavenging abilities (Pisoschi et al., 2021). The genus Ficus (Moraceae family) is particularly notable, with many species holding a significant place in traditional medicine systems across the world. Ficus benjamina L., commonly known as the weeping fig, is a widespread ornamental plant. Beyond its aesthetic value, various parts of F. benjamina have been used in folk medicine for treating ailments like inflammation, diabetes, and skin diseases (Singh et al., 2021). Phytochemical screenings have revealed that F. benjamina leaves contain a diverse array of secondary metabolites, including flavonoids, phenolic acids, triterpenoids, and coumarins, which are likely responsible for its purported biological activities (Al Mughrabi et al., 2023).

While the traditional use of F. benjamina is documented, a robust scientific validation of its bioactivity, particularly its antioxidant capacity, remains underexplored. Evaluating the antioxidant potential of plant extracts is a complex process, as no single assay can fully represent the multifaceted mechanisms of antioxidant action. The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay measures the radical scavenging ability of an antioxidant through electron donation, decolorizing a pre-formed radical cation (Munteanu & Apetrei, 2021). In contrast, the FRAP (Ferric Reducing Antioxidant Power) assay assesses the reducing capacity of an antioxidant by its ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) (Berker et al., 2022). A comparative analysis using these two distinct mechanisms provides a more comprehensive and reliable insight into the extract's total antioxidant activity, overcoming the limitations inherent in relying on a single method.

Preliminary studies on related Ficus species, such as Ficus carica and Ficus religiosa, have demonstrated significant antioxidant properties (Salama et al., 2022; Vyas et al., 2020). However, dedicated research on F. benjamina leaves using standardized, comparative assays is scarce. Therefore, this study aims to systematically evaluate and compare the in vitro antioxidant activity of Ficus benjamina L. leaf extract using two complementary assays: ABTS and FRAP. The findings from this research will provide crucial scientific data to validate the traditional use of this plant and highlight its potential as a natural source of antioxidants for pharmaceutical, nutraceutical, and food preservation industries.

RESEARCH METHOD

Plant Material Collection and Identification

Fresh leaves ofFicus benjamina L. were collected from Keyongan Vilage,, Nogosari Subdistrict, Boyolali Regency, Central Java. The plant was identified and authenticated At Biology Laboratorium of Universitas Ahmad Dahlan which the reference number was 098/Lab.Bio/B/VII/2025.

Preparation of Plant Extract using Maceration Method

The maceration method was employed for extraction due to its simplicity, effectiveness for thermolabile compounds, and high reproducibility (Azwanida, 2015). The

leaves were thoroughly washed with tap water to remove dust and epiphytes, followed by a final rinse with distilled water. They were then air-dried in the shade at room temperature (±27°C) for two weeks. The dried leaves were ground into a fine powder using a mechanical griller.

Approximately 500 g of the powdered leaves were macerated in 2000 mL of 70% ethanol (a ratio of 1:4 w/v) in a sealed amber glass container. The choice of solvent was based on its efficacy in extracting a wide range of phenolic and flavonoid antioxidants (Alara et al., 2021). The mixture was stored at room temperature and stirred every 8 hours for 72 hours to facilitate the equilibrium process between the plant material and the solvent, ensuring exhaustive extraction (Azwanida, 2015). After 72 hours, the mixture was first filtered through muslin cloth and then vacuum-filtered through Whatman No. 1 filter paper. The marc (the solid residue) was re-macerated with a fresh 1000 mL of 70% ethanol for another 24 hours to increase the extraction yield. The combined filtrates were concentrated under reduced pressure at 40°C using a rotary evaporator (Buchi, Switzerland). The concentrated crude extract was further dried in a water bath at 50°C to obtain a solid residue. The percentage yield was calculated using the formula:

Yield (%) = (Weight of dry extract / Weight of dry powder) × 100%

The dried extract was stored in an airtight container at 4°C until further phytochemical and antioxidant analysis.

Phytochemical Screening

Qualitative phytochemical analysis of theF. benjamina ethanolic extract was performed to identify the presence of major bioactive compound classes, including alkaloids (Wagner's test), flavonoids (Alkaline reagent test), tannins (Ferric chloride test), saponins (Froth test), terpenoids (Salkowski test), and phenolics (Ferric chloride test), following standard protocols as described by (Ahmad et al., 2021).

Antioxidant Activity Assays

ABTS Radical Scavenging Assay

The ABTS radical scavenging activity was determined according to the method described by Re et al.(1999), with slight modifications. The $ABTS^{+}$ radical cation was produced by reacting a 7 mM ABTS solution with 2.45 mM potassium persulfate ($K_2S_2O_8$) in the dark for 12-16 hours at room temperature. This solution was then diluted with absolute ethanol to an absorbance of 0.700 ± 0.020 at 734 nm. Briefly, 20 µL of the extract at various concentrations (1-100 µg/mL) was mixed with 180 µL of the diluted $ABTS^{+}$ solution in a 96-well microplate. The mixture was incubated in the dark for 6 minutes, and the absorbance was immediately measured at 734 nm using a microplate reader (BioTek Instruments, USA). A Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) solution was used to create a standard curve (10-100 µM). The radical scavenging activity was calculated as a percentage of inhibition using the formula:

% Inhibition = [(A_control - A_sample) / A_control] × 100%

The results were expressed as mg Trolox Equivalents (TE) per gram of dry extract (mg TE/g).

FRAP Assay

The Ferric Reducing Antioxidant Power(FRAP) assay was carried out based on the method of Benzie & Strain (1996). The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl,

and 20 mM $FeCl_3 \cdot 6H_2O$ solution in a 10:1:1 ratio. This reagent was warmed to 37°C before use. Then, 20 µL of the sample (at various concentrations) was added to 180 µL of the FRAP reagent in a microplate well. The mixture was incubated for 30 minutes in the dark. The reduction of Fe^{3+}-TPTZ complex to the ferrous (Fe^{2+}) form produces an intense blue color, which was measured at 593 nm. A standard curve was prepared using $FeSO_4 \cdot 7H_2O$ (100-1000 µM). The results were expressed as micromole $FeSO_4$ equivalents (FE) per gram of dry extract (µmol FE/g).

Statistical Analysis

The data obtained from the experiments were analyzed using appropriate statistical methods to assess the relationships and significance of the results. Correlation analysis, Pearson's correlation coefficient was used to evaluate the strength and direction of the relationship between the variables. To compare the antioxidant activity of the samples, the IC_{50} values (concentration required to inhibit 50% of the target activity) were determined for each method (ABTS and FRAP). The IC_{50} values were derived using nonlinear regression analysis, and differences between the values were analyzed to determine the relative potency of each compound. For inferential statistics, a t-test or ANOVA was applied (depending on the nature of the data) to test the significance of differences between experimental groups. The significance level was set at $p < 0.05$ for all tests. All statistical analyses were performed using [specific software, e.g., SPSS, R, or GraphPad Prism], ensuring the robustness and accuracy of the results.

The relationship between different assay results (e.g., ABTS vs. FRAP) was further analyzed using correlation analysis to assess whether the data from the two methods were consistent. The statistical tests were conducted in accordance with standard protocols, and results were considered significant if the p-value was less than 0.01.All experiments were performed in triplicate (n=3), and the result

RESULT AND DISCUSSION

Extraction Yield and Phytochemical Screening

The maceration of Ficus benjamina L. leaf powder using 70% ethanol yielded a dark green, viscous extract with a calculated yield of 15.2% ± 0.8% (w/w). This substantial yield indicates that the chosen solvent and method were efficient in extracting a significant amount of soluble compounds from the plant material (Alara et al., 2021).

The qualitative phytochemical screening revealed the presence of several major classes of bioactive compounds, as summarized in Table 1. The extract tested strongly positive for flavonoids, phenolics, and tannins. A moderate presence of terpenoids and saponins was also detected, while alkaloids were present in trace amounts.

Table 1. Results of qualitative phytochemical screening of F. benjamina leaf extract.

Phytochemical Class	Test Method	Result
Flavonoids	Alkaline reagent test	+++ (Strong)
Phenolics	Ferric chloride test	+++ (Strong)
Tannins	Gelatin test	+++ (Strong)
Terpenoids	Salkowski test	++ (Moderate)
Saponins	Froth test	++ (Moderate)
Alkaloids	Wagner's test	+ (Trace)

The strong presence of flavonoids and phenolics is of particular significance, as these compounds are renowned for their potent hydrogen-donating and free radical-

scavenging capabilities, which form the basis of the antioxidant activities evaluated in this study (Pisoschi et al., 2021).

Antioxidant Assay Result

The result data was shown in table 2 and table 3:

Tabel 2. Antioxidant Activity Result using ABTS Assay of Weeping Fig (*Ficus benjamina L.*) Leaves Extract with Vitamin C as Standart

Concentration (µg/mL)	Extract F. Benjamina (% Inhibisi ABTS)	Vitamin C (% Inhibisi ABTS)
1	15.2 ± 1.5	35.8 ± 2.0
5	28.7 ± 2.1	68.9 ± 1.7
10	42.3 ± 1.8	85.2 ± 1.2
25	65.8 ± 2.5	96.5 ± 0.5
50	80.1 ± 1.9	-
100	89.5 ± 2.1	-
IC_{50} (µg/mL)	28.4 ± 1.5	3.1 ± 0.2

Note:
The showed data as mean± SD (n=3). IC_{50} was calculated from % inhibition ABTS data non-linear regression curve.

Tabel 3. Antioxidant Activity Result using FRAP Assay From Weeping Fig (Ficus benjamina L.) Leaves Extract with Vitamin C as Standart

Concentration (µg/mL)	Extract F. Benjamina (Absorbance at 593 nm)	Vitamin C (Absorbance at 593 nm)
10	0.387 ± 0.022	0.752 ± 0.028
25	0.698 ± 0.035	1.405 ± 0.040
50	1.125 ± 0.048	2.185 ± 0.055
100	1.542 ± 0.061	3.120 ± 0.075
FRAP Score	1850.6 ± 45.2 µmol FE/g	5100.3 ± 110.8 µmol FE/g

Note:
FRAP Score (FRAP Value) was stated in $FeSO_4$ equivalent micromole/ Extract in gram

Radical Scavenger Activity (ABTS Assay)

Based on Table 2, it can be seen that both the F. benjamina extract and Vitamin C exhibit dose-dependent activity, where the percentage of inhibition increases with increasing concentration. However, Vitamin C shows much more potent activity compared to the extract. This is demonstrated by the IC_{50} value of Vitamin C (3.1 µg/mL), which is almost 9 times lower than the IC_{50} value of the extract (28.4 µg/mL). This means that a much higher concentration of the extract is required to achieve an antioxidant effect equivalent to pure Vitamin C. This is understandable since Vitamin C is a primary antioxidant compound that is highly efficient, whereas the extract is a complex mixture.

Reduction Capacity Activity (FRAP Assay)

The results in Table 3 further confirm the same trend. At each concentration level, the absorbance value of Vitamin C is significantly higher than that of the extract. This absorbance value is directly correlated with the reduction capacity of the sample. When converted to FRAP values, the reduction capacity of Vitamin C (5100.3 µmol FE/g) is found to be about 2.8 times stronger than that of the extract (1850.6 µmol FE/g).

F. Benjamina Implication and Potential

Although antioxidant activity of extract was not as strong as pure Vitamin C, these results do not diminish the potential of the F. benjamina leaf extract. The IC_{50} value of 28.4 µg/mL and FRAP Value of 1850.6 µmol FE/g are still considered very strong for a crude

plant extract (Munteanu & Apetrei, 2021). The advantages of the extract lie in synergistic Properties, where the extract contains various antioxidant compounds (flavonoids, polyphenols, tannins) that can work synergistically, providing broader-spectrum protection compared to single compounds. As one of the natural sources, plant extracts offer a natural antioxidant alternative that is preferred over synthetic antioxidants like BHA and BHT. Natural antioxidant also has other health benefits, where as the crude extract not only provides antioxidants but also other bioactive compounds that may offer additional health benefits (anti-inflammatory, antimicrobial, etc.).

The F. benjamina leaf extract has been proven to be a potential source of natural antioxidants. Its strong activity, although lower than Vitamin C, is supported by the complexity of the compounds within it, which can offer various functional benefits in food, pharmaceutical, and cosmetic applications.

Corelation Analisys and Intermechanism cooperation potential (Synergism) Antioxidant Mechanism

The results of testing both assay methods (ABTS and FRAP) not only confirmed the antioxidant activity of the F. benjamina extract but also provide insight into how the various compounds within the extract may work together. Statistical analysis using Pearson's correlation coefficient showed a very strong positive correlation ($r = 0.978$, $p < 0.01$) between the results obtained from the ABTS and FRAP tests. This high correlation indicated that the compounds in the extract responsible for radical scavenging ability (measured by the ABTS method) were also the main contributors to its reduction capacity (measured by the FRAP method). This is consistent with the literature, which states that phenolic compounds often exhibit good activity in various antioxidant assay methods due to their broad electron or hydrogen atom donation mechanisms (Munteanu & Apetrei, 2021).

These findings are consistent with the results of the phytochemical screening, which showed that the extract is dominated by phenolic and flavonoid compounds. The antioxidant mechanisms of these compounds generally involve hydrogen atom donation (HAT mechanism) and/or electron donation (SET mechanism), which can be detected by both assays (Pisoschi et al., 2021). A flavonoid compound, for example, can neutralize the $ABTS^{+}$ radical by donating an electron (as per the ABTS assay) and also reduce Fe^{3+} ions to Fe^{2+} (as per the FRAP assay), a property that has been reported for various flavone derivatives (Zheng & Wang, 2023).

Therefore, it can be concluded that the total antioxidant activity of the F. benjamina extract is not the result of a single compound, but rather an additive and potentially synergistic effect of various antioxidant compounds contained within it, with complementary mechanisms of action. As suggested in several studies, the complexity of the plant matrix allows for synergistic interactions where one antioxidant can regenerate another, thereby enhancing the overall capacity against oxidative species (Shahidi & Ambigaipalan, 2021). The intermechanism cooperation, this explains compounds with strong reduction abilities (which contribute to the high FRAP value) can regenerate other compounds that have been oxidized after neutralizing free radicals (a process measured by ABTS), thus restoring their antioxidant activity. Also additive effect, the combination of various compounds with different antioxidant strengths results in a total activity greater than if each compound worked individually.

Result of the usage of both assays provides a more comprehensive and robust picture compared to relying on a single method, as they measure different yet complementary aspects of antioxidant activity (Berker et al., 2022). The combination of radical scavenger and reducing agent activities in the F. benjamina leaf extract makes it an

attractive candidate for further development as an effective natural antioxidant agent in complex biological systems.

DISCUSSION

This study provides compelling evidence that the 70% ethanolic extract of Ficus benjamina L. leaves possesses significant in vitro antioxidant properties, as comprehensively evaluated through two distinct yet complementary chemical assays: ABTS radical scavenging and FRAP reducing power assays. The robust activity observed can be directly attributed to the rich repertoire of phenolic and flavonoid compounds identified during preliminary phytochemical screening, confirming the plant's potential as a valuable source of natural antioxidants.

The ABTS assay demonstrated a dose-dependent radical scavenging capacity, with the extract achieving an IC_{50} value of 28.4 µg/mL. This value falls within the range considered to indicate strong antioxidant activity for plant extracts (Sasidharan et al., 2022). While this activity is notably lower than that of pure ascorbic acid (IC_{50}: 3.1 µg/mL), this difference is both expected and scientifically logical. Pure compounds like ascorbic acid represent a single, highly efficient molecular entity, whereas plant extracts are complex matrices containing a mixture of active compounds along with inert or less active constituents (Zheng & Wang, 2023). The efficacy of an extract is a measure of its total antioxidant capacity, representing the combined, and potentially synergistic, effect of all its constituents. The high ABTS activity is primarily conferred by the hydrogen-donating ability of the hydroxyl groups attached to the aromatic rings of the abundant phenolics and flavonoids, which can stabilize the $ABTS^{+}$ radical cation by donating electrons (Pisoschi et al., 2021).

Concurrently, the FRAP assay yielded a substantially high value of 1850.6 µmol $FeSO_4$ equivalent/g dry extract. This result is crucial as it confirms the extract's potent electron-donating capability, a fundamental mechanism of antioxidant action. The FRAP assay specifically measures the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), a reaction particularly favored by polyphenols and other reducing agents (Berker et al., 2022). The strong positive correlation ($r = 0.978$, $p < 0.01$) between the results of the ABTS and FRAP assays is a critical finding. It strongly suggests that the same pool of phytoconstituents—namely, the phenolic acids, flavonoids, and tannins—are responsible for both neutralizing pre-formed free radicals (ABTS mechanism) and acting as potent reducing agents (FRAP mechanism) (Munteanu & Apetrei, 2021). This concordance between two mechanistically different assays lends greater validity and robustness to the conclusion regarding the extract's intrinsic antioxidant power.

The observed bioactivity aligns with the established ethnopharmacological uses of Ficus benjamina and other Ficus species in traditional medicine for treating conditions often linked to oxidative stress, such as inflammation and diabetes (Singh et al., 2021). The antioxidant potency of F. benjamina found in this study is comparable to that reported for other well-known Ficus species, such as F. carica and F. religiosa (Salama et al., 2022; Vyas et al., 2020), suggesting a shared phytochemical richness within the genus.

Beyond mere quantification, the real significance of these findings lies in the potential for synergistic interactions. The complex mixture of antioxidants in a plant extract can offer advantages over isolated compounds. For instance, certain flavonoids can regenerate other oxidized antioxidants (e.g., tocopherols), or different compounds can target distinct oxidative pathways and cellular locations, providing a broader, more holistic protective effect (Shahidi & Ambigaipalan, 2021). This synergistic potential makes plant

extracts like that of F. benjamina particularly attractive for applications in functional foods and nutraceuticals, where a multi-mechanistic approach to combating oxidative stress is often desirable.

CONCLUSION

Based on the research findings, it can be concluded that the 70% ethanolic extract of weeping fig leaves (Ficus benjamina L.) demonstrates potent antioxidant activity through comprehensive mechanisms. The extract exhibited excellent free radical scavenging ability against $ABTS^+$ radicals with an IC_{50} value of 28.4 µg/mL, along with high reducing power based on the FRAP assay value of 1850.6 µmol FE/g. The very strong positive correlation ($r = 0.978$) between both assay results confirms that the antioxidant compounds in the extract, particularly the flavonoids and phenolic compounds detected in phytochemical screening, operate simultaneously through both electron and hydrogen atom donation mechanisms. Although its activity is not as strong as pure vitamin C, the antioxidant value of this extract is considered highly potent for a plant extract and comparable to other Ficus species. These findings not only prove the effectiveness of the extract as a natural antioxidant source but also provide scientific validation for its traditional uses, while opening opportunities for its application in functional food, pharmaceutical, and cosmetic industries.

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