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PHYTOCHEMICAL ANALYSIS AND TOTAL ANTIOXIDANT CAPACITY OF MULBERRY LEAF (MORUS ALBA L.)

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Abstract

The mulberry plant (Morus alba L.) is one of the herbal plants that are useful as a phytopharmaceutical. Mulberry leaves are known to be rich in antioxidants that can overcome infections caused by bacteria, fungi, protozoa, antimalaria, and anticancer. This study used a descriptive approach with experimental studies using *in vitro* techniques and bioassays using *Artemia salina* larvae. *In vitro* techniques were used in phytochemical tests, total phenolic tests with Folin-Ciocalteu reagent, and total antioxidant capacity tests using DPPH, ABTS, and FRAP reagents. The research data were analyzed using GraphPad Prism 10 software. The main phytochemicals in mulberry leaves include flavonoid derivatives and phenolic compounds with high antioxidant activity. The total phenolic content, expressed as gallic acid equivalents, was 16,24 mg GAE/g DW. Antioxidant capacity measured by the ABTS method yielded an IC_{50} value of 22,42 µg/mL, the DPPH assay showed an IC_{50} value of 343,413 µg/mL, while the FRAP assay indicated an IC_{50} value of 24,578 µg/mL.

Keywords : mulberry leaves, antioxidant, phytochemical, free radicals

Abstrak

Tanaman Murbei (Morus alba L.) merupakan salah satu tanaman herbal yang bermanfaat sebagai tanaman fitofarmaka. Daun murbei diketahuil kaya akan kandungan antioksidan yang tinggi dapat mengatasi infeksi akibat bakteri, jamur, protozoa, antimalaria, dan antikanker. Penelitian ini menggunakan pendekatan deskriptif dengan studi eksperimental menggunakan Teknik in vitro dan bioassay dengan menggunakan larva Artemia salina. Teknik in vitro digunakan pada uji fitokimia, uji fenolik total dengan pereaksi folin-cio calteu dan uji kapasitas total antioksidan yang menggunakan pereaksi DPPH, ABTS, dan FRAP, data penelitian dianalisis menggunakan aplikasi graphpad prism 10. Kandungan fitokimia utama dalam daun murbei terdapat derivat flavonoid dan senyawa fenolik daun murbei yang tinggi kadar antioksidan. Kadar fenolik total diperoleh hasil standar asam galat 16,24 mg GAE/g DW. Uji kapasitas total antioksidan menggunakan metode ABTS ekstrak daun murbei didapatkan nilai IC_{50} 22,42 µg/mL, uji kapasitas total dengan metode DPPH ekstrak daun murbei didapatkan nilai IC_{50} 343,413 µg/mL, dan uji kapasitas dengan metode FRAP ekstrak daun murbei nilai IC_{50} 24,578 µg/mL.

Kata kunci : *daun murbei, antioksidan, fitofarmaka, radikal bebas*

INTRODUCTION

In 2024, Indonesia ranked 15th as the country with the highest level of air pollution in the world (IQAir, 2024). High levels of pollution make Indonesians vulnerable to exposure to free radicals. Free radicals are unstable and highly reactive molecules or atoms that can trigger oxidative stress and damage vital biomolecules such as DNA, proteins, and lipids. The damage to biomolecules contributes to the pathogenesis of various diseases, including inflammation. (Chandimali, 2025).

The mulberry plant (Morus alba L.) is traditionally used by the community in South Sulawesi Province to treat cancer. Additionally, mulberry is also utilized to address infections caused by bacteria, fungi, and protozoa. It's leaves are widely recognized in traditional medicine across various cultures due to their active ingredient content, including antioxidants, vitamins, minerals, and bioactive compounds such as alkaloids, anthocyanins, and flavonoids (Mustanir, 2019).

The increase in free radicals resulting from anticancer (antineoplastic) therapy exacerbates oxidative stress, which can damage cellular components such as proteins, lipids, and DNA. Oxidative stress arises from a homeostatic imbalance caused by excessive production of Reactive Oxygen Species (ROS) within the body. In Indonesia, public knowledge and understanding of the benefits of mulberry leaves remain limited, necessitating education on their use as a multibeneficial herbal substance.

Many plants are known to have therapeutic benefits, but some also contain toxic compounds. Therefore, evaluating the toxicity of herbal extracts is crucial to ensure their safety as medicinal ingredients. The objective of this research is to further investigate the antioxidant capacity and safety of mulberry leaves (*Morus alba L.)* as well as to determine their secondary metabolite content, antioxidant levels, and total phenolic content. Furthermore, the results of this research can provide a scientific basis for biodiversity conservation and the development of phytopharmaceutical products based on local wisdom. This research topic provides an important foundation for utilizing herbal ingredients as raw materials for natural medicines to prevent disease and support health.

Antioxidants suppress the production of reactive oxygen species (ROS) by inhibiting the formation of hydroperoxides (ROO) and hydrogen peroxide (H_2O_2), as well as neutralizing other free radicals. Among the most prevalent causes of nosocomial infections is *Staphylococcus* aureus, which is implicated in the pathogenesis of mastitis, dermatitis (skin inflammation), toxic shock syndrome, respiratory tract infections, and food poisoning.

METHODS

This study uses an experimental laboratory study and mulberry leaf extract (Morus alba L.). The mulberry leaf extract will undergo in vitro testing, including phytochemical analysis, phenolic assessment, and total antioxidant capacity evaluations using DPPH, ABTS, and FRAP reagents. The samples utilized are fresh, unoxidized

mulberry leaves sourced from plantations in Cisoka, Tangerang Regency, Banten, Indonesia. A total of 300 grams of mulberry leaves were collected and dried at room temperature for 2 – 3 days, avoiding direct sunlight. After drying, the weight of the mulberry leaves was reduced to 250 grams, which were then ground using a blender, yielding 75 grams of powdered simplicia.
The percolation process began with the preparation of the apparatus, including a percolation funnel, filter paper, and cotton wool. The filter paper and cotton were positioned to cover the funnel's interior surface, after which the macerated material was transferred into the device. Methanol was added as the solvent until the sample was submerged 1-2 cm below the solvent surface. The funnel was sealed and allowed to stand for a 24-hour incubation period. Afterward, the extract was eluted at a rate of 30-50 drops per minute, with a continuous supply of methanol maintained to keep the solvent level 1-2 cm above the sample. Collection continued until the resulting effluent was colorless. Finally, the extract was evaporated to produce a concentrated crude extract with a pasta-like consistency.

A Gallic acid calibration curve was established to determine the total phenolic content. A stock solution of Gallic acid (5 mg/mL) was prepared by dissolving 0,25 g of Gallic acid in 5 mL of 95% methanol, then diluting to a final volume of 50 mL with distilled water. From this stock, aliquots of 6, 8, 10, 12, and 14 mL were transferred into separate flasks and diluted to 100 mL to obtain a series of working standards at concentrations of 300, 400, 500, 600, and 700 µg/mL, respectively.
For the assay, 0,2 mL of each standard solution was mixed with 15,8 mL of distilled water and 1 mL of Folin-Ciocalteu reagent. The mixture was allowed to stand for 8 minutes, after which 3 mL Na_2CO_3. The solution was then homogenized and incubated at room temperature for 2 hours. The absorbance was subsequently measured at a wavelength of 767 nm using a Genesys 30 *Visible Spectrophotometer.*
Determination of Total Phenolic Content. Mulberry leaf extract (0,4 g) was dissolved to a final volume of 10 mL using a methanol-water mixture. An aliquot of 0,2 mL of this extract solution was pipetted into a reaction vessel and mixed with 15,8 mL of distilled water and I mL of *Folin-Ciocalteu* reagent. The mixture was thoroughly mixed and allowed to stand for 8 minutes in the dark. Subsequently, 3 mL of a 20% Na_2CO_3 solution was added, and the mixture was incubated at room temperature for 2 hours. The development of a blue color indicated the reaction's progress. The absorbance of the solution was then measured at 765 nm using a Genesys 30 *visible spectrophotometer.* The total phenolic content was quantified based on the pre-established standard calibration curve.

The DPPH scavenging assay was conducted using DPPH reagent and sample stock solutions, with Trolox serving as the reference standard. Absorbance was measured using a *Genesys 30 visible spectrophotometer* at the maximum absorption wavelength determined from the control absorbance.

Determination of Antioxidant Activity using the DPPH Method. The sample stock solution was prepared by dissolving 0,04 g of the mulberry leaf sample in 20 mL of methanol. From this stock, a series of

dilutions was prepared to achieve concentrations of 100, 200, 300, 400, 500, and 600 µg/mL. Each was diluted to a final volume of 10 mL with methanol. Subsequently, 3,5 mL DPPH reagent was dispensed into separate test tubes, to which 0.5 mL of each diluted sample was added. The mixtures were homogenized and incubated in a water bath at 37˚C for 30 minutes in the dark. The reaction was indicated by the color degradation from deep purple to yellow. The assay was performed in triplicate for each sample concentration. The absorbance was then measured at a wavelength of 516 nm, which was predetermined as a control absorbance using a *visible spectrophotometer.*

The ABTS solution was prepared by dissolving 7,1 mg of ABTS in 5 mL of ethanol. Separately, 3,5 mg of potassium persulfate was dissolved in 5 mL of distilled water. Both solutions were then incubated in the dark for 12 hours. Following incubations, the solutions were mixed and diluted to a final volume of 25 mL of distilled water. The absorbance of this working solution was measured at 734 nm and adjusted to reach an absorbance value between 0,7 and 0,8 (±0,02). The control solution was prepared by taking 1 mL of the diluted ABTS working solution. The absorbance was then recorded at 734 nm Genesys 30 *visible spectrophotometer.*
A sample stock solution was prepared by dissolving 0,01 g of mulberry leaf extract in 19 mL of distilled water. The stock solution was subsequently diluted to obtain working concentrations of 10, 20, 30, 40, and 50 µg/mL. For the assay, 250 µL of each sample concentration was added to a test tube containing 250 µL of the ABTS working solution. This procedure was performed in triplicate. Additionally, Trolox was used as a reference standard, with the same procedure as the samples. The absorbance of both the samples and Trolox standards was measured at 734 nm using a Genesys 30 *visible spectrophotometer.*

The FRAP reagent was prepared by synthesizing three separate stock solutions. First, 187 mg of sodium acetate trihydrate was mixed with 16 mL of acetic acid and diluted to a final volume of 250 mL with distilled water. In a separate flask, 150 mg of TPTZ was dissolved in 40 mM HCL to reach a volume of 50 mL. Additionally, 270 mg of ferric chloride hexahydrate ($FeCl_3.6H_2O$) was dissolved in distilled water and diluted to 100 mL.

The working FRAP reagent was then prepared by mixing 25 mL of the sodium acetate trihydrate solution, 2,5 mL of the TPTZ solution, and 2,5 mL of the $FeCl_3.6H_2O$ solution. This mixture was subsequently diluted to a final volume of 100 mL using distilled water.

The BSLT (Brine Shrimp Lethality Test) is conducted using hatched *Artemia salina* larvae. The hatching process involves seawater, an aerator, and a light source. *Artemia salina* eggs are placed in seawater with aeration and allowed to hatch for 1 to 2 days under a heat source provided by the light. After 1 to 2 days, the eggs will hatch, and the *Artemia salina* larvae are ready for use. The BSLT is performed using a duplicate technique with various extract concentrations as follows: 1000 µg/mL, 800 µg/mL, 600 µg/mL, 400 µg/mL, 200 µg/mL, and 100 µg/mL. The sample preparation involves taking 0,02 g of mulberry leaf extract, adding yeast, and

dissolving in 1000 µL of DMSO until mixed thoroughly.

Subsequently, 20 mL of seawater is added, and each tube is prepared with 1000 µL, 800 µL, 600 µL, 400 µL, 200 µL, and 100 µL of the extract, with 2 tubes per concentration. Each tube is then diluted with seawater to a final volume of 1000 µL. Following this, 1000 µL of seawater containing 10 larvae is added to each tube. The tubes are allowed to stand for 24 hours, after which the number of dead larvae is recorded.

RESULTS

Mulberry leaves obtained directly from plantations in the Balaraja area, Tangerang Regency, Banten. Before drying, 300 grams of mulberry leaves were selected and dried for 2-3 days indoors without direct sunlight exposure. The mulberry leaves shrank by 50 grams, resulting in 250 g of dried leaves. The dried mulberry leaves were ground using a blender, yielding 75 gram of simplisia. Next, the mulberry leaves were extracted and then percolated, yielding 9,58 grams of extract, resulting in a yield of 12,77%.

$$Persentase\ rendemen\ (yield) = \frac{berat\ ekstrak}{berat\ simplisia} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$= \frac{9{,}58\ gram}{75\ gram}\ x\ 100\% = 12{,}77\%$$

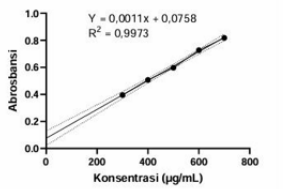
Qualitative phytochemical testing of mulberry leaf extract with several other reagents showed that mulberry leaves contain alkaloids, anthocyanins, cardyglycosides, coumarins, flavonoids, glycosides, phenolics, quinones, saponins, and steroids. They tested negative for betacyanin.

Fitokimia	Ekstrak Daun Murbei	Reagen/Metode
Alkaloid	+	Mayer, Dragendorff
Antosianin	+	NaOH
Betasianin	-	NaOH
Kardioglikosida	+	Keller Killiani
Koumarin	+	NaOH
Flavonoid	+	NaOH
Glikosida	+	Borntrager
Fenolik	+	Folin Cioceteau
Kuinon	+	H_2SO_4
Saponin	+	Foam
Steroid	+	Liebermann Burchard
Tanin	+	$FeCl_3$
Terpenoid	+	Liebermann Burchard

Phytochemical Testing of Mulberry Leaf Extract.

Determination of Gallic Acid Standards. Gallic acid in various concentrations was diluted to obtain its absorbance values. The data obtained was used to create a standard curve, resulting in the standard curve equation for glycolic acid: Y=0,0011x + 00758, with absorbance as the Y axis and concentration as the X axis, with R^2=0,9973.

Konsentrasi Asam Galat (µg/mL)	Rata – rata Absorbansi
300	0,395 ± 0,16
400	0,507 ± 0,005
500	0,597 ± 0,005
600	0,727 ± 0,005
700	0,818 ± 0,005



Standard Acid Test Yield Curve.

Phenolic Test of Mulberry Leaf Extract. Mulberry leaf extract was reacted with Folin-Ciocalteu reagent and read using a *visible spectrophotometer.* The absorbance results were entered into the equation Y=0,0011x + 0,0578, and obtained from the standard curve of gallic acid, with the absorption value of mulberry leaf extract as Y and the X value as the phenolic content. For a phenolic value of 484,72 µg/mL with

a dilution of 2x. Thus, the total phenolic content was 969,44 µg/mL. The total phenolic concentration was expressed as mg gallic acid equivalents per gram of dried weight (mg GAE/g DW). The result was 16,24 mg GAE/g DW with the formula:

$$C = C1 \times (\frac{V}{M})$$

$$C = 0,48472 \frac{mg}{mL} \times (\frac{10\ mL}{0,3\ gram})$$

$$C = 16,24\ mg\ \frac{GAE}{g} DW$$

Keterangan rumus:

C = Total senyawa fenolik (mg GAE/g DW)
C1 = Kadar total fenolik (mg/ mL)
V = Volume pelarut ekstrak (mL)
M = Massa Kering Ekstrak (gram)

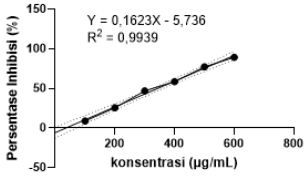
Tabel 4. 3. Absorbansi dan Kadar Fenolik Total Ekstrak Daun Murbei

Rata – rata Absorbansi	Kadar Fenolik Total (µg/mL)	Kadar Fenolik Pengenceran 2x (µg/mL)	Kadar Total Fenolik (mg GAE/g DW)
1. 0,603 2. 0,609	484,72	969,44	16,24

Absorbance and Total Phenolic Content of Mulberry Leaf Extract

Mulberry leaf extracts at various concentrations were reacted with DPPH reagent at a maximum wavelength of 516 nm and a control absorbance of 0,623 using a *visible spectrophotometer* to obtain the test absorbance. From the absorbance obtained, the average results were used to obtain the inhibition percentage. The percentage inhibition was used as the basis for creating a linear equation curve, Y=0,1623x – 5.736, with R^2 of 0,9939, with the percentage inhibition as the Y axis and the concentration as the X axis, resulting in an IC_{50} value of 343,413 µg/mL.

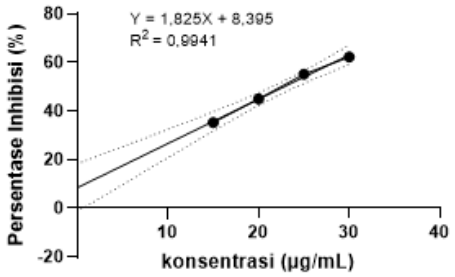
Konsentrasi Ekstrak (µg/mL)	Absorbansi Rata – rata	Persen Inhibisi (%)	IC_{50}
100	0,567 ± 0,003	8,989 ± 0,576	
200	0,464 ± 0,008	25,522 ± 0,394	
300	0,331 ± 0,003	46,870 ± 0,585	343,413
400	0,258 ± 0,004	58,587 ± 0,715	
500	0,142 ± 0,002	77,207 ± 0,431	
600	0,067 ± 0,002	89,246 ± 0,435	



Total Antioxidant Capacity Curve DPPH Method.

Mulberry leaf extract was reacted with ABTS reagent and examined using a *visible spectrophotometer* with a wavelength of 734 nm and a control absorbance value of 0,411. The test absorbance was obtained, then the average was obtained, and the percentage of inhibition was calculated. From the data obtained, a linear equation curve was created with a value of Y=1,825x + 8,395 and an R^2 value of 0,9941, resulting IC_{50} value of 22,42 µg/mL.

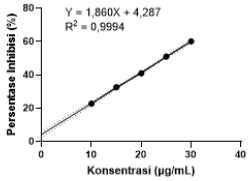
Konsentrasi (µg/mL)	Ekstrak Rata – rata Absorbansi	Persen Inhibisi	IC50
15	0,266 ± 0,008	35,280 ± 1,987	
20	0,226 ± 0,005	45,012 ± 1,296	
25	0,184 ± 0,004	55,231 ± 0,960	22,428
30	0,155 ± 0,006	62,287 ± 1,483	



Total Antioxidant Capacity Curve ABTS Method.

Mulberry leaf extract was reacted with FRAP reagent in various concentrations and examined using a *visible spectrophotometer,* and the absorbance value at a wavelength of 594 nm was obtained for the absorbance test. Then, the average absorbance of each concentration was calculated to determine the percentage of inhibition. From the results obtained, a linear equation curve with the value Y=1,860x + 4,287 and an R^2 value of 0,9994 was obtained, resulting in an IC_{50} value of 24,578 µg/mL.

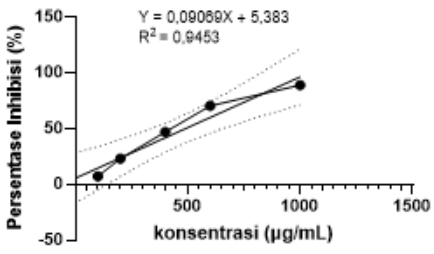
Konsentrasi Ekstrak (µg/mL)	Absorbansi Rata – rata	Persen Inhibisi (%)	IC_{50}
10	0,123 ± 0,004	22,764 ± 2,687	
15	0,141 ± 0,004	32,624 ± 2,049	
20	0,173 ± 0,004	40,963 ± 1,459	24,578
25	0,202 ± 0,005	50,970 ± 1,218	
30	0,238 ± 0,007	60,084 ± 1,230	



Total Antioxidant Capacity FRAP Method.

Toxicity Test using the BSLT Method. The number of larvae that survived and died within 24 hours was counted to obtain the mortality percentage. The mortality percentage of larvae at various concentrations of mulberry leaf extract and concentration logs was used to find the linear equation curve. The results obtained from the linear equation curve Y=0,09069x + 5,383 with an R^2 value of 0,9453, with the Y axis as the percentage of mortality and the X axis as the log concentration, resulted in an LC_{50} value of 491,972 µg/mL.

Konsentrasi ekstrak daun murbei (µg/mL)	Log Konsentrasi	Persen Mortalitas
100	2,00	7,018
200	2,30	22,917
400	2,60	46,667
600	2,78	70,213
1000	3,00	88,679



BSLT method of Mulberry Leaf Extract.

DISCUSSION

Phytochemical Test of Mulberry Leaf Extract. In this study, the results showed that the results of mulberry leaf extract contained alkaloid compounds, anthocyanins, cardioglycosides, coumarins, flavonoids, glycosides, phenolics, quinones, saponins, steroids, tannins, and terpenoids. This is in line with research by Pulungan et al (2022). In his research, the results showed that there were positive results in an alkaloid test with Dargendorff and Wagner reagents in the methanol extract from mulberry leaves. In this study, flavonoids, steroids, and terpenoids, saponins were also reported. This research was conducted by Abeysinghe et al.

Steroids were detected in extracts with non-polar solvents such as hexane and dichloromethane, but were also detected in extracts with polar solvents such as ethanol, water, and methanol. This suggests that some types of steroids or their derivatives have polar groups or conjugated forms (e.g., glycosylation) that increase their solubility in polar solvents (Kholidah, 2020). The diversity of phytochemical compounds in murbei leaves indicates that murbei leaves are a potential source of natural bioactive compounds with therapeutic potential.

Total phenolic content is a key parameter in assessing the antioxidant capacity of plants. High total phenolic content generally indicates high antioxidant capacity. In this study, total phenolic content was measured using the Folin-Ciocalteu method, in which phenolic compounds reduce the Folin-Ciocalteu reagent under alkaline conditions, forming a blue complex with a maximum absorbance at 765 nm. The absorbance value is directly proportional to the total phenolic content and is expressed as gallic acid equivalents (GAE).

Test Total Phenolic Levels of Mulberry Leaf Extract. The phenolic test carried out on the methanol extract from mulberry leaves in this study obtained a total phenolic compound of 484,72 µg/mL or the equivalent of 12,64 mg GAE/g DW. These results are in line with the study conducted by Abeysinghe on phenolic levels from the methanol extract of mulberry leaves, which had a yield of 52.6 mg GAE/g DW. Compared with commercial products, the phenolic content was low at 35,2 mg GAE/g DW, so from the results of this classification, it can be said that the phenolic levels from both studies were high. According to Mohammed et al, this result is because methanol levels can increase the solubility of non-phenolic components due to the presence of water molecules in organic compounds, so that the test results for phenolic levels are higher than those of extracts with other solvents. Phenolic levels in a plant are influenced by several factors, one of which is the agrochemical characteristics of the soil where it is nursery, climatic conditions, agricultural and harvesting technology, and plant varieties.

Test the total antioxidant capacity in this study, to test the ability of mulberry leaf methanol extract compared to the ability of Trolox to inhibit free radicals from ABTS. The IC_{50} level of mulberry leaves was 22,248 µg/mL with an R^2=0,9941 and the IC_{50} level in the Trolox was obtained as 13,27 µg/mL with a value of R^2=0,9987. From the results of this research, it can be concluded that the total antioxidant capacity in Trolox is lower than that of the mulberry leaf methanol extract. IC_{50} values can be influenced by the type and temperature of the solvent, as was researched. His research found the results of IC_{50} values using the ABTS method were 21,32±0,03 mg/mL with 50% ethanol solvent at room temperature (˷30̊ C), 17,19±0,02 mg/mL with hot water solvent (100̊C), and 19,56±0,07 mg/mL with room temperature water solvent (˷30̊ C).

This study was conducted on a total antioxidant capacity test to test the ability of mulberry leaf methanol extract compared to the ability of trolox to ward of DPPH free radicals. The IC_{50} level of mulberry leaves was obtained as 343,413 µg/mL with a value of R_2 = 0,9939. Both can be categorized as moderate antioxidants. According to Rahayu et al., antioxidant strength is grouped based on the results of the IC_{50} value into very strong antioxidants if <150 µg/mL, strong if the IC_{50} level is 150 - 300 µg/mL, moderate if the IC_{50} level is 300 - 400 µg/mL, and weak if the IC_{50} level is 400 – 500 µg/mL. In another study conducted by Siti Fatimah Hanum in 2022, an IC_{50} value was obtained from mulberry leaf methanol extract, which was not much different, namely 31,81 µg/mL, so it could be said to be a very strong antioxidant in the range <50. Apart from that, in other studies, the IC_{50} value of mulberry leaf methanol extract was also obtained, namely 28,85 µg/mL strong range >50.

In this study, a FRAP test was carried out to test the ability of antioxidants in mulberry leaf extract to release electrons. From the results of this study, an IC_{50} of mulberry leaf extract of 24,578 µg/mL was obtained with an R_2 value of 0,9994. From the IC_{50} Trolox results, it was 10,54 µg/mL with an R_2 value of 0,9968. According to Ghasemi et al, there are differences in geographical conditions and climate change conditions, temperature, altitude and diversity of natural vegetation in the area which can

cause differences in the bioactive components of a plant.

The results obtained in research conducted by Muslim examined the FRAP level of mulberry leaf methanol extract from the Gowa Regency area, South Sulawesi, with an IC_{50} result of 13,146 µg/mL. Other research also conducted by Jurian from the University of Jember was obtained at 43,95%, and research from the Kupang area, NTT, with an IC_{50} value of 8,35 µg/mL. This difference in results can be influenced by several factors, one of which is time. In research conducted by Soural et al, it was found that ongoing reactions can be seen from detailed measurements over time using the FRAP method. Some phenolic compounds do not react optimally in a short time, so they will show a significant increase when read again sometime later. In his research, the absorbance results of FRAP solutions will increase over time, namely around 0,002 per hour at laboratory temperature; After a 4-hour interval, the value changes to around 0,001 per hour.

In the toxicity test, 10 Artemia salina larvae were added to various concentrations of mulberry leaf extract, and the percentage of death was calculated within 24 hours, so that an LC_{50} of mulberry leaf extract was obtained of 491,972 µg/mL. These results are in line with research obtained by Nordin et al, the higher the concentration of mulberry leaf extract used, the greater the cytotoxic effect, reaching 250 µg/mL of 98,37±0,62%. According to Surya et al, in their research, it was found that LC_{50} levels were classified as very toxic if ≤ 30 µg/mL, toxic 31 – 1000 µg/mL, and non-toxic ≥ 1000 µg/mL. It can be concluded that the results in both studies are classified as toxic according to the classification from Surya et al.

Limitations of Mulberry Leaf Research:

1. Variation in Plant Growing Conditions
   The bioactive compound content of mulberry leaves is strongly influenced by environmental factors such as soil type, climate, altitude, leaf age, and harvest season. This study did not fully control for all these factors.
2. Limitations in the number and origin of samples. Mulberry leaf samples were only taken from specific locations to be representative of all mulberry leaf varieties grown in other regions.
3. Limitations in the extraction or processing method.
   The extraction/processing method used has the potential to affect the levels and activity of active compounds. Using a single method does not allow for comparison of effectiveness with other methods.
4. limitations in test parameters
   This study only tested certain parameters (e.g., antioxidant activity, antibacterial activity, or flavonoid content); other biological effects of mulberry leaves have not been fully explored.
5. Limitations of in-depth compound analysis
   This study has not specifically identified all active compounds using advanced techniques such as HPLC, GC-MS, or LC-MS/MS.

CONCLUSION

The methanol extract of Morus alba leaves shows strong potential as a natural antioxidant due to its high phenolic content (16.24 mg GAE/g DW) and notable antioxidant activity in ABTS, DPPH, and FRAP assays. Phytochemical screening confirmed the presence of multiple bioactive compounds, although betacyanin was absent. However, the extract was classified as toxic in the BSLT assay (LC50 491.72 µg/mL). Therefore, further in vivo studies, solvent variation research, and exploration of other plant parts are recommended to fully evaluate its antioxidant benefits and safety.

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