International Jurnal Islamic Education, Research and Multiclturalism (IJIERM)
Available online https://journal.yaspim.org/index.php/IJIERM/index

IN-VITRO ANTIOXIDANT ACTIVITY OF EGGPLANT (*Epicarp Solanum Melongena* L) SKIN EXTRACT ON AFLATOXIN-B1-INDUCED RATS

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Abstrak

Kulit terong adalah bagian luar dari buah terong (Solanum melongena L) yang mengandung berbagai senyawa bioaktif dan telah diakui memiliki aktivitas terapeutik seperti anti-inflamasi, anti-oksidan, anti-virus, anti-bakteri, dan anti-kanker. Aktivitas-aktivitas ini menjadikan kulit terong sebagai sumber alami antioksidan yang berpotensi untuk membantu mengurangi stres oksidatif dan mencegah kerusakan seluler. Penelitian ini bertujuan untuk mengevaluasi potensi antioksidan ekstrak etanol kulit terong melalui analisis fitokimia dan aktivitas antioksidan menggunakan metode 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) dan 2,2-Diphenyl-1-picrylhydrazyl dengan mengukur konsentrasi penghambatan setengah maksimum (IC50). Selain itu, penelitian ini juga menguji pengaruhnya terhadap enzim-enzim yang berhubungan dengan stres oksidatif pada tikus yang diinduksi aflatoksin B1, termasuk malondialdehid, peroksidasi lipid 4-hidroxynonenal, serta mengevaluasi kadar superoksida dismutase dalam serum tikus. Hasil penelitian menunjukkan bahwa ekstrak etanol kulit terong memiliki sifat antioksidan yang signifikan, efektif dalam menetralkan radikal bebas. Aktivitas antioksidan ekstrak etanol kulit terong, yang dinilai melalui metode 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) dan 2,2-Diphenyl-1-picrylhydrazyl, menunjukkan potensi antioksidan yang kuat dengan nilai konsentrasi penghambatan setengah maksimum yang menjanjikan. Dalam uji in-vivo, ekstrak etanol kulit terong menunjukkan efek perlindungan terhadap stres oksidatif, yang mengarah pada penurunan kadar malondialdehid dan peroksidasi lipid 4-hidroxynonenal serta peningkatan aktivitas superoksida dismutase pada tikus yang diinduksi aflatoksin B1. Dosis optimal ekstrak etanol kulit terong dalam penelitian ini adalah 600 miligram per kilogram berat badan, yang memberikan hasil terbaik dalam menurunkan kadar malondialdehid dan peroksidasi lipid 4-hidroxynonenal serta meningkatkan aktivitas superoksida dismutase, menjadikannya kandidat antioksidan alami yang menjanjikan.

Kata Kunci: Ekstrak Terong; Antioksidan; Stres Oksidatif; Aflatoksin; Radikal Bebas

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Article History	Submitted: 24 January 2025	Accepted: 21 February 2025	Published: 25 February 2025

Abstract

Eggplant skin is the outer part of the eggplant fruit (Solanum Melongena L), which contains various bioactive compounds and has been recognized for its therapeutic activities such as anti-inflammatory, anti-oxidant, anti-viral, anti-bacterial, and anti-cancer properties. These properties make eggplant skin a potential natural source of antioxidants that may help in reducing oxidative stress and preventing cellular damage. This study aims to evaluate the antioxidant potential of ethanol extract of eggplant skin through phytochemical analysis and antioxidant activity using 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and 2,2-Diphenyl-1-picrylhydrazyl methods by assessing the half maximal inhibitory concentration. Additionally, the study examines its effect on oxidative stress-related enzymes in aflatoxin B1-induced rats, including malondialdehyde, lipid peroxidation 4-hydroxynonenal, and evaluates superoxide dismutase levels in rat serum. The results demonstrated that the ethanol extract of eggplant peel possesses significant antioxidant properties, effectively neutralizing free radicals. The antioxidant activity of ethanol extract of eggplant skin, as assessed through 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and 2,2-Diphenyl-1-picrylhydrazyl methods, showed strong antioxidant potential with promising half maximal inhibitory concentration values. In the in-vivo test, aubergine skin ethanol extract exhibited a protective effect against oxidative stress, leading to a reduction in malondialdehyde and lipid peroxidation 4-hydroxynonenal levels while enhancing superoxide dismutase activity in aflatoxin B1-induced rats. The optimal dose of aubergine skin ethanol extract in this study was determined to be 600 milligrams per kilogram of body weight, which provided the best results in lowering malondialdehyde and lipid peroxidation 4-hydroxynonenal levels and enhancing superoxide dismutase activity, making it a promising natural antioxidant candidate.

Keywords: *Eggplant Extract; Antioxidant; Oxidative Stress; Aflatoxin; Free Radicals*

INTRODUCTION

Free radicals and reactive oxygen species (ROS) have been identified as major contributors to various health disorders in humans. An imbalance between the generation and neutralization of these pro-oxidants triggers oxidative stress, which damages critical biomolecules such as lipids, proteins, and DNA. This damage is associated with the development of chronic diseases, including cancer, diabetes, premature aging, and other degenerative conditions. To counteract these harmful effects, human cells produce enzymatic antioxidants such as superoxide dismutase and

catalase, alongside non-enzymatic compounds like ascorbic acid, tocopherol, and glutathione (Engwa, 2018). However, external factors, including exposure to mycotoxins found in food and agricultural products, can exacerbate oxidative stress. Mycotoxins are secondary metabolites produced by toxigenic fungi, particularly Aspergillus flavus and Aspergillus parasiticus, which generate aflatoxin (AF). These toxic compounds pose significant health risks, as exposure can lead to acute and chronic poisoning, organ damage, and in severe cases, mortality in both humans and animals (Faradila Rahmadani Asdar et al., 2024; Omotayo et al., 2019).

Aflatoxin AF is classified as a class I carcinogenic substance by the International Agency for Research on Cancer (Suryani et al., 2023), highlighting its severe toxicological effects. AF is hepatotoxic, nephrotoxic, mutagenic, and immunotoxic, with toxicity linked to the generation of free radicals that trigger lipid peroxidation. This oxidative process primarily affects polyunsaturated fatty acids in cell membranes, leading to extensive DNA damage and severe biological dysfunction (Yilmaz et al., 2018). Oxidative stress induced by AF contributes to multiple pathophysiological conditions, making mitigation strategies essential. One widely adopted approach is the use of antioxidants as food additives, which serve to prevent oxidative degradation while also inhibiting toxigenic mold growth and mycotoxin production (Neeff et al., 2018). The exploration of natural antioxidants has gained significant attention, as they offer protective effects with minimal side effects. Among these, eggplant (Solanum melongena L.), a commonly consumed vegetable, exhibits considerable potential due to its rich bioactive composition and well-documented health benefits (Nandana et al., 2023).

Eggplant has long been used in traditional Chinese medicine for its therapeutic properties, including its anti-inflammatory, antiviral, antibacterial, and anticancer effects (Hilmarni et al., 2024). Research has identified bioactive components such as amides and phenylpropanoids, which contribute to its strong antioxidant and radical-scavenging activities (Wulandari, 2023). Specific compounds, including N-trans-p-coumaroyltyramine, N-trans-feruloyltyramine, and N-trans-feruloyloctopamine, play a critical role in its antioxidant mechanisms (Ratri et al., 2022). Additionally, phenylpropanoid derivatives like neochlorogenic acid have demonstrated substantial antioxidant activity when assessed through ABTS, DPPH, and FRAP assays (Lelario et al., 2019; Song et al., 2021). The combination of these compounds enhances the ability of

eggplant to mitigate oxidative stress, making it a promising candidate for counteracting the detrimental effects of AF exposure. Studies continue to explore the mechanisms through which these bioactive compounds function, particularly in preventing lipid peroxidation and oxidative DNA damage, ultimately supporting eggplant's therapeutic role in disease prevention.

To further investigate the protective effects of eggplant, in vitro and in vivo studies have been conducted. The in vitro approach utilizes DPPH free radical capture and ABTS coloration methods to assess antioxidant activity. Meanwhile, in vivo testing involves male Wistar rats exposed to aflatoxin B1, with key parameters including lipid peroxidation markers such as LPO-4HNE and MDA, as well as antioxidant enzyme levels like SOD. Results from these studies indicate that ethanol extracts derived from eggplant skin exhibit strong protective potential against oxidative stress and aflatoxin-induced damage (Rahmawati et al., 2021; Retnowati et al., 2022). These findings reinforce the therapeutic benefits of eggplant, particularly in preventing degenerative diseases associated with oxidative stress. By leveraging natural antioxidants, such as those found in eggplant, future research may develop effective dietary interventions to reduce the harmful impact of mycotoxin exposure and support overall health maintenance.

THEORETICAL BASIS

Free radicals are unstable molecules due to unpaired electrons, making them highly reactive and capable of damaging DNA, proteins, and lipids (Theafelicia & Narsito Wulan, 2023). Research on free radicals began in the 20th century when Moses Gomberg synthesized triphenylmethyl (Di Meo & Venditti, 2020). Sources include endogenous factors like cell metabolism, inflammation, and aging, as well as exogenous factors such as pollution, radiation, and cigarette smoke. Mitochondria are the main site of ROS production, involving enzymes like NADPH oxidase, xanthine oxidase, and cyclooxygenase (Phaniendra et al., 2015). Oxidative stress arises from an imbalance between free radical production and the body's antioxidant defenses, leading to cellular damage (Widiastuti, 2022). Its consequences include lipid peroxidation, protein modification, and DNA damage, contributing to chronic diseases such as diabetes, cardiovascular diseases, cancer, and neurodegenerative disorders (Emekdar et al., 2023; Hartini et al., 2024; Nurmaida et al., 2024).

The eggplant plant is not only known as a food ingredient but also offers various therapeutic benefits, including analgesic, antipyretic, antioxidant, anti-inflammatory, anti-asthmatic, hypolipidemic, hypotensive, and antiplatelet activities, as well as the ability to lower intraocular pressure and prevent anaphylaxis (Rusli, 2022). This plant thrives at temperatures between 70 and 85 degrees Fahrenheit, growing to a height of 2 to 4 feet with downy, star-shaped leaves and stems, and small, star-shaped purple flowers. Its fruit has a shiny, edible skin and is rich in bioactive compounds, particularly antioxidants, which contribute to its medicinal properties. These antioxidants help neutralize free radicals, which are key contributors to oxidative stress in the body. Additionally, eggplant contains phenolic compounds, flavonoids, and anthocyanins, which have been linked to various health benefits, including improved cardiovascular health, neuroprotection, and anti-cancer properties, making it a valuable dietary component.

Oxidative stress occurs when the balance between free radical production and the capacity of the body's antioxidant system is disturbed. In normal amounts, reactive oxygen species (ROS) serve important functions in cell signaling and other physiological processes. However, excessive ROS levels due to factors such as inflammation, infection, cancer, or aging can lead to oxidative damage, including lipid peroxidation, protein modification, and DNA damage. This damage plays a role in the development of various chronic diseases, such as diabetes, cardiovascular disease, cancer, and neurodegenerative disorders (Guo et al., 2023). Eggplant contains antioxidants, including flavonoids, anthocyanins, and other phenolic compounds, which help combat the harmful effects of free radicals (Firmansyah & Duppa, 2022). By neutralizing ROS, eggplant may reduce oxidative stress, repair cellular damage, and lower the risk of chronic diseases. Its bioactive compounds further support immune function and anti-inflammatory responses, enhancing its therapeutic potential in disease prevention and management.

Lipid peroxidation is an oxidative process that occurs in unsaturated fatty acids, proceeding through three main phases: initiation, propagation, and termination. Free radicals attack carbon-carbon bonds in lipids, leading to the formation of end products such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE) (Khaira et al., 2024; Valgimigli, 2023). The process begins when free radicals abstract a hydrogen atom from a methylene group in an unsaturated fatty acid, generating a lipid radical. This radical subsequently reacts with molecular oxygen, forming peroxyl

radicals that continue the chain reaction, leading to hydroperoxide production. The accumulation of lipid peroxidation products is not only cytotoxic but can also serve as signaling molecules that influence gene expression and cell survival. Additionally, excessive lipid peroxidation has been linked to various pathological conditions, including neurodegenerative diseases, cardiovascular disorders, and cancer, highlighting the need for antioxidants to mitigate its detrimental effects.

The antioxidant defense system plays a crucial role in combating oxidative stress (Ni Wayan Oktarini A. C., 2020). Antioxidants are classified into enzymatic and non-enzymatic types. Endogenous antioxidants, such as superoxide dismutase, catalase, and glutathione peroxidase, directly neutralize free radicals and are found in organelles like mitochondria, endoplasmic reticulum, lysosomes, and cytosol. Exogenous antioxidants from fruits and vegetables further enhance the body's defense system. Their mechanisms include preventing free radical formation, neutralizing reactive species, and repairing oxidized molecules (Martemucci et al., 2022; Tcanty Indrianti et al., 2024). Increased intake of exogenous antioxidants may protect against oxidative damage (Delvinie Angelia Kusdianty et al., 2024). However, antioxidants have dual roles—beneficial at low levels but potentially harmful at high concentrations. Compounds like polyphenols, flavonoids, and vitamin E show therapeutic potential. Strategies to manage oxidative stress involve lifestyle changes, antioxidant-rich diets, and therapeutic interventions targeting oxidative mechanisms (Kaushik et al., 2020).

To address the complexity of oxidative stress, recent studies have developed multidimensional strategies, including preventive and interventional approaches. Preventive strategies focus on lifestyle modifications such as antioxidant-rich diets, regular exercise, and stress management (Forman & Zhang, 2021). Consuming foods like green vegetables, berries, nuts, and traditional herbs can significantly reduce free radical exposure. Clinical research suggests that antioxidant combinations from natural sources are more effective than single supplements due to synergistic phytochemical interactions (Li et al., 2014). Advanced strategies include targeted therapies using nanotechnology and genetic engineering. Antioxidant nanoparticles allow precise delivery of antioxidants to cells experiencing oxidative damage. Additionally, gene therapy aimed at modifying endogenous antioxidant enzymes, such as superoxide dismutase and catalase, is being explored to prevent degenerative diseases. These innovative approaches seek to enhance the body's antioxidant defense

system efficiently, potentially reducing the risk of chronic diseases caused by oxidative stress (Polaka et al., 2022).

RESEARCH METHODS

This study examines the antioxidant activity of eggplant skin ethanol extract using experimental methods to determine the relationship between the independent variable, ethanol extract of eggplant skin, and the dependent variable, which includes a decrease in serum LPO-4HNE and MDA levels, as well as an increase in serum SOD levels. The research was conducted at Cendikia Laboratory and Cendikia Phytochemical Laboratory between April and June 2024. A total of 50 male Wistar rats (150–200g) were acclimatized for one week at a room temperature of 22–25°C under a 12-hour light-dark cycle and were fed pellets with Na-CMC drinking water (Lie et al., 2018). Ethical clearance approval was obtained from the Research Ethics Commission of the Faculty of Medicine, Prima Indonesia University, under letter number 034/KEPK/UNPRI/I/2025. For extract preparation, ±600 grams of eggplant skin powder was macerated in 96% ethanol (1:3 b/v) for seven days with stirring at 200–250 rpm for ±48 hours. The extract was filtered and evaporated using a rotary evaporator at 45–50°C, followed by further processing with a water bath to remove residual solvent. The simplia was examined macroscopically, organoleptically, and microscopically, including tests for ash content, moisture content, and soluble compounds.

Antioxidant activity was analyzed using the DPPH and ABTS methods. Ethanol extract solutions of eggplant skin at varying concentrations (10–80 µg/mL) were mixed with ABTS solution, incubated for six minutes in a dark room, and measured using a UV-Visible spectrophotometer at 734 nm. The assay was performed three times, with ascorbic acid as a comparator. The IC50 value was determined from a regression equation to assess antioxidant effectiveness. In vivo testing involved male Wistar rats adapted for 14 days before treatment. They were divided into groups and given AFB1 induction along with Eggplant Peel Ethanol Extract at doses of 200, 400, and 600 mg/200 g body weight. On the final day, biochemical parameters such as SOD, MDA, and LPO enzyme

activity were analyzed. Blood samples were centrifuged, and the serum was used for further analysis. Data were processed using SPSS 22 with descriptive statistical methods. The Shapiro-Wilk test assessed normality, and homogeneity was determined before performing One-Way ANOVA, followed by Tukey's Post Hoc test if significant differences were found. This statistical approach ensured the validity of research findings on the antioxidant potential of eggplant skin extract.

The research flow chart can be seen in the following figure:

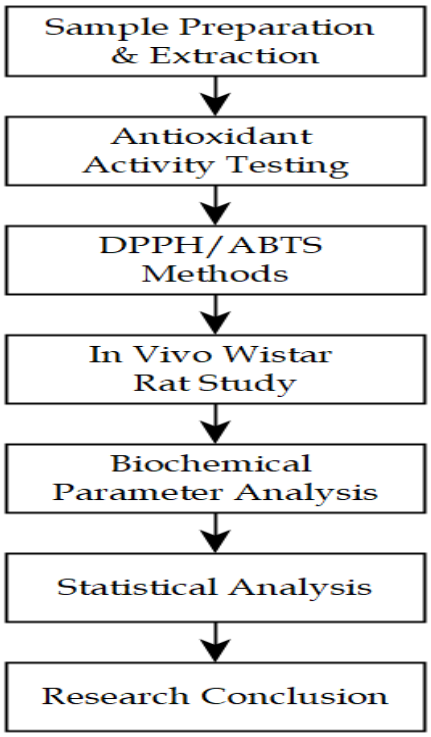

Figure 1: Research flow chart

RESULTS AND DISCUSSION

Results

1. Phytochemical Test Results of ethanol extract of skin

Table 1. Phytochemical screening of ethanol extract of eggplant peel

No.	Parameters	Results

1	Alkaloids	+
2	Flavonoids	+
3	Saponins	+
4	Tannins	+
5	Glycosides	+
6	Steroids/Triterpenoids	+

The phytochemical test results indicate that the ethanol extract of eggplant peel contains various bioactive compounds, including alkaloids, flavonoids, saponins, tannins, glycosides, and steroids/triterpenoids. These compounds possess diverse pharmacological properties, such as antioxidant, antibacterial, and anti-inflammatory activities. The presence of flavonoids in the extract aligns with findings in bay leaves, which contain flavonoid compounds such as myristin, quercetin, rutin, and luteolin. Flavonoids play a crucial role in scavenging free radicals and enhancing biological activity in the body. In this study, the screening of ethanol extract of bay leaves was conducted using a qualitative method with coloring, a common phytochemical analysis technique that identifies specific compounds based on color changes. Thus, this study contributes to the identification of bioactive compounds with potential applications in health and pharmaceutical fields, providing a foundation for further research into natural therapeutic agents.

2. DPPH Examination Results on Ethanol Extract of Eggplant Peels

The DPPH (α,α-diphenyl-β-picrylhydrazyl) assay is used to evaluate the antioxidant activity of ethanol extract of eggplant peels. DPPH is a stable free radical that appears purple in solution, but when it interacts with antioxidants, it undergoes a reduction reaction, turning into hydrazine yellow due to hydrogen donation from the substrate (Azzahra & Indradi, 2021). The absorbance values and percentage of radical scavenging activity were measured at various extract concentrations. The mean percentage of inhibition was 103.333%. Absorbance values at different concentrations were recorded, with inhibition percentages exceeding 100%. The assessment included concentration variations ranging from 0 to 32 µg/ml,

with corresponding absorbance and damping percentage values. Total values for concentration, damping percentage, and calculated variables were also obtained. The collected data were used to determine the relationship between concentration and absorbance, which was further analyzed based on recorded values. The values obtained for each concentration point reflect measured absorbance levels and percentage damping, contributing to the dataset generated in this examination.

Table 2. Assessment of Absorbance and percentage of silencing in Ethanol DPPH Examination of Ethanol Extract of Eggplant Peels

Concentration µg/ml	Absorbance	Percent Damping	X	Y	XY	X2
0	0.6840	0	0	0	0	0
2	-0.1390	120.322	2	120.322	240.643	4
4	-0.1400	120.468	4	120.468	481.871	16
8	-0.1390	120.322	8	120.322	962.573	64
16	-0.1380	120.175	16	120.175	1922.81	256
32	-0.1360	119.883	32	119.883	3836.26	1024
Total			62	601.17	7444.15	1364
Mean			103.333	100.195	1240.69	227.333

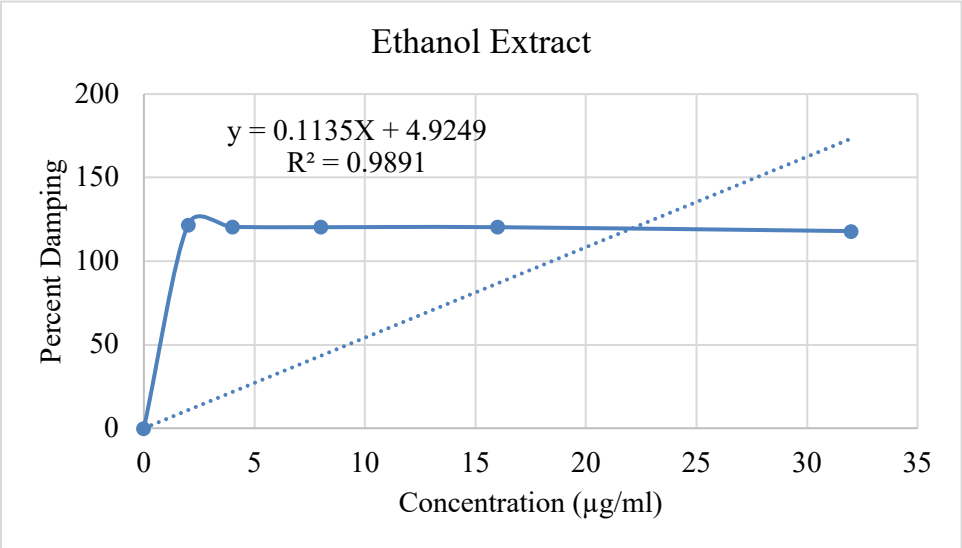

Figure 2. Percent Reduction Graph of ethanol extract of eggplant peel

The concentration of antioxidant activity tested using the α,α-diphenyl-β-picrylhydrazyl (DPPH) assay; C18H12N5O6, M=394.33, is expressed by the parameter IC50, which represents the inhibition concentration-50. This value indicates the concentration of antioxidants required to reduce the DPPH radical concentration by 50%. A lower IC50 value corresponds to higher antioxidant activity. The calculation involves plotting an inhibition curve based on absorbance values at different concentrations. The IC50 value is categorized into four levels: very strong (<50 µg/mL), strong (50–100 µg/mL), moderate (101–150 µg/mL), and weak (151–200 µg/mL). Antioxidants with lower IC50 values exhibit greater radical scavenging ability, which is influenced by factors such as concentration, solubility, and reactivity with free radicals. The determination of IC50 provides a quantitative measure of antioxidant capacity, allowing comparisons between different samples. The inhibition curve serves as a reference for evaluating antioxidant efficiency based on the tested concentrations.

3. Results of ABTS Examination on Ethanol Extract of Eggplant Peels

Table 3. Absorbance Assessment and Percentage of Reduction in Ethanol ABTS Examination of Ethanol Extract of Eggplant Peels

Concentration µg/ml	Absorbance	Percent Damping	X	Y	XY	X2
0	0.6840	0	0	0	0	0
2	-0.1490	1.217.836	2	1.217.836.257	2.435.672.515	4
4	-0.1400	1.204.678	4	1.204.678.363	481.871.345	16
8	-0.1390	1.203.216	8	1.203.216.374	9.625.730.994	64
16	-0.1390	1.203.216	16	1.203.216.374	1.925.146.199	256
32	-0.1230	1.179.820	32	1.179.824.561	3.775.438.596	1024
Total			62	600.877.193	7.388.596.491	1364
Mean			103.333	1.001.461.988	1.231.432.749	227.333

The ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] assay is used to evaluate the antioxidant activity of ethanol extract of eggplant peels. This method measures the ability of antioxidants to scavenge ABTS radicals, which results in a change in absorbance. The reaction occurs through electron transfer, leading to the reduction of ABTS radicals and a decrease in absorbance values. Absorbance and percentage inhibition values were recorded at various concentrations ranging from 0 to 32 µg/mL. The IC50 value, representing the concentration required to inhibit 50% of ABTS radicals, serves as a parameter for antioxidant classification. The IC50 value is categorized as very strong (<50 µg/mL), strong (50–100 µg/mL), moderate (101–150 µg/mL), and weak (151–200 µg/mL) (Utami, 2017). The inhibition percentage was obtained by comparing absorbance values at different concentrations. The assessment of IC50 provides a reference for determining antioxidant capacity based on the reduction of ABTS radicals.

4. ABTS Testing Results for Ascorbic Acid

According to Torres et al. (2018), ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] is an organic cation radical compound used to measure antioxidant activity. The reaction occurs at pH 7.4 and is assessed based on time and percentage of discoloration as a function of concentration. The ABTS radical cation undergoes a color change from blue or green to colorless when reduced by antioxidants. The Trolox Equivalent Antioxidant Capacity (TEAC) method evaluates the ability of antioxidants to donate proton radicals, leading to stability. This method allows for antioxidant measurement in both aqueous and lipid phases, making it widely applicable. The ABTS assay is considered simple, rapid, and effective for assessing antioxidant activity in various systems. The percentage of discoloration provides a measurable parameter to quantify the extent of antioxidant interaction. The reaction kinetics and absorbance values obtained from ABTS measurements serve as indicators of radical scavenging efficiency, which is determined by evaluating changes in absorbance at specific wavelengths.

Table 4. Absorbance Assessment and Percentage of Reduction in Ethanol ABTS Examination of Ethanol Extract of Eggplant Peels

Concentration µg/ml	Absorbance	Percent Damping	X	Y
0	0.7240	0	0	0
2	-0.1330	118.37	2	118.37
4	-0.1330	118.37	4	118.37
8	-0.1290	117.818	8	117.818
16	-0.1280	117.68	16	117.68
32	-0.1280	116.851	32	116.851
Total			62	589.088
Mean			103.333	981.814

5. Results of DPPH Examination of Ascorbic Acid

Table 5. Absorbance Assessment and Percentage of Reduction in Ethanol DPPH Examination of Ascorbic Acid

Concentration µg/ml	Absorbance	Percent Damping	X	Y
0	0.6840	0	0	0
2	-0.1340	119.591	2	119.591
4	-0.1350	119.737	4	119.737
8	-0.1370	120.029	8	120.029
16	-0.1380	120.175	16	120.175
32	-0.1410	120.614	32	120.614
TOTAL			62	600.146
Mean			10.3333	100.024

The IC50 value categorizes antioxidant activity as very strong (<50 µg/mL), strong (50–100 µg/mL), moderate (101–150 µg/mL), and weak (151–200 µg/mL) (Utami, 2017). The presence of N-trans-coumaroyltyramine, N-trans-feruloyltyramine, and N-trans-feruloyloctopamine in Solanum melongena contributes to radical scavenging activity, while neochlorogenic acid exhibits antioxidant

potential in vitro using ABTS, DPPH, and FRAP methods (Song et al., 2021). The ethanol extract of Solanum melongena Linn fruit has been analyzed as a strong in vitro antioxidant. Mbah et al. (2019) reported that it scavenged DPPH and nitric oxide, inhibited lipid peroxidation, and reduced ferric ions based on FRAP analysis, showing activity comparable to vitamin C and EDTA. The antioxidant potential was dose-dependent, meaning its effectiveness increased with concentration. The presence of bioactive compounds in Solanum melongena plays a crucial role in its radical scavenging properties. Various studies highlight the significance of phenolic and flavonoid compounds in contributing to its antioxidant activity.

6. Results of *In-Vivo* Examination of Free Radical and Antioxidant Levels

Table 6. MDA Examination Results

Group	Induction	Test material	Mean ± SD ng/mL
Neutral	-	-	5.42 ± 0.006
neg control	H1	-	15.42 ± 0.006
control post+1	H1	H5	6.38 ± 0.006
post+2 control	H5	H1	5.57 ± 0.009
Treatment 1	H1	H5	9.87 ± 0.009
Treatment 2	H5	H1	9.03 ± 0.009
Treatment 3	H1	H5	8.97 ± 0.006
Treatment 4	H5	H1	7.25 ± 0.009
Treatment 5	H1	H5	6.33 ± 0.009
Treatment 6	H5	H1	5.55 ± 0.009

Table 6 presents the results of the Malondialdehyde (MDA) level examination as an indicator of oxidative stress in various treatment groups. The "Neutral" group had the lowest average MDA level of 5.42 ± 0.006 ng/mL, while the negative control group showed an increase in MDA levels up to 15.42 ± 0.006 ng/mL. The post+1 and post+2 control groups had lower MDA levels compared to the negative control, at 6.38 ± 0.006 ng/mL and

5.57 ± 0.009 ng/mL, respectively. The treatment groups showed a reduction in MDA levels compared to the control group, with values ranging from 5.55 ± 0.009 ng/mL to 9.87 ± 0.009 ng/mL. These results indicate that the administration of test materials may contribute to reducing oxidative stress. Overall, this data suggests that the treatment groups have the potential to lower free radical levels as measured by MDA levels.

Table 7. LPO-4HNE Examination Results

Group	Induction	Test material	Mean ± SD µm
Neutral	-	-	0.13 ± 0.009
neg control	H1	-	15.01 ± 0.009
control post+1	H1	H5	2.68 ± 0.014
post+2 control	H5	H1	1.25 ± 0.012
Treatment 1	H1	H5	5.03 ± 0.011
Treatment 2	H5	H1	4.23 ± 0.011
Treatment 3	H1	H5	3.12 ± 0,011
Treatment 4	H5	H1	3.05 ± 0012
Treatment 5	H1	H5	2.64 ± 0.012
Treatment 6	H5	H1	1.10 ± 0.012

The neutral group showed very low levels of LPO-4HNE, reflecting a baseline condition without lipid peroxidation and minimal oxidative damage. This indicates that, in the absence of external stressors, lipid peroxidation does not occur significantly. In contrast, the negative control group exhibited significantly elevated LPO-4HNE levels, suggesting severe oxidative stress and extensive lipid damage. The positive control group demonstrated a moderate reduction in LPO-4HNE levels compared to the negative control, signifying a partial protective effect in mitigating lipid peroxidation. The various treatment groups showed a decrease in LPO-4HNE levels compared to the negative control. Treatment 1 had lower LPO-4HNE levels than the negative control but remained higher than the positive control. Treatment 2 and Treatment 3 showed further reductions, while Treatment 4 and Treatment 5 exhibited even lower levels. Treatment

6 had the lowest LPO-4HNE levels among the treatment groups, approaching values observed in the neutral group.

Table 8. SOD Examination Results

Group	Induction	Test material	Mean ± SD ng/mL
Neutral	-	-	8.97 ± 0.009
neg-1 control	H1	-	2.11 ± 0.011
control post+1	H1	H5	8.05 ± 0.011
post+2 control	H5	H1	8.69 ± 0.009
Treatment 1	H1	H5	5.99 ± 0.009
Treatment 2	H5	H1	6.57 ± 0.011
Treatment 3	H1	H5	6.98 ± 0.009
Treatment 4	H5	H1	7.69 ± 0.011
Treatment 5	H1	H5	8.15 ± 0.009
Treatment 6	H5	H1	8.97 ± 0.011

Table 7 shows the results of SOD (Superoxide Dismutase) analysis in the various experimental groups. The "Neutral" group represented a baseline condition with minimal lipid peroxidation, indicated by low LPO-4HNE levels of 8.97 ± 0.009 ng/mL. The "neg-1 control" and "pos+1 Th control" groups showed high levels of LPO-4HNE, 2.11 ± 0.011 ng/mL and 8.05 ± 0.011 ng/mL, respectively, indicating significant oxidative stress and increased lipid peroxidation. The various treatment groups ("treatments 1-6") showed lower LPO-4HNE levels than the negative control. "Treatment 1" and "Treatment 2" had moderately reduced LPO-4HNE levels, while "Treatment 3" and "Treatment 4" exhibited further decreases. "Treatment 5" demonstrated a more pronounced decline, and "Treatment 6" had the lowest LPO-4HNE levels among all treatments, at 8.97 ± 0.011 ng/mL, closely resembling the neutral group. Differences in LPO-4HNE levels across treatments indicate variations in oxidative stress reduction.

Discussion

Phytochemical screening revealed that the ethanol extract of eggplant skin contains several bioactive compounds, including alkaloids, flavonoids, saponins, tannins, glycosides, and steroids/triterpenoids. These compounds play crucial roles in biological activity, particularly as natural

antioxidants. The screening process utilized a qualitative staining method to identify active compounds within the extract. Flavonoids, specifically quercetin and rutin, are notable contributors to the extract's free radical scavenging capacity. The presence of these compounds serves as a preliminary indicator of the extract's potential effectiveness as an antioxidant agent. The comprehensive screening approach helped establish a foundation for understanding the extract's chemical composition and its potential therapeutic applications. The identification and characterization of these bioactive compounds provide valuable insights into the potential mechanisms through which the extract may exert its therapeutic effects. Further detailed analysis of the individual compounds could help optimize their extraction and application in pharmaceutical formulations.

The antioxidant activity of eggplant skin ethanol extract was evaluated using the DPPH method, which relies on the color change of DPPH free radicals from purple to yellow when reduced by antioxidants. The extract's potency was quantified using the IC50 value, which represents the concentration required to reduce 50% of DPPH free radicals. A lower IC50 value indicates stronger antioxidant activity. The research findings demonstrated that the eggplant skin ethanol extract possessed an IC50 value that classified it in the strong antioxidant category. This significant result highlights the extract's efficient free radical scavenging capabilities and reinforces its potential as a valuable source of natural antioxidants. The standardized DPPH method provides reliable and reproducible results, making it an excellent tool for comparing antioxidant potency across different studies and extracts. The strong antioxidant activity observed suggests potential applications in both pharmaceutical and nutraceutical industries.

The ABTS method was employed as an additional approach to assess the antioxidant activity of eggplant skin ethanol extract. This method involves monitoring the decolorization of the green-blue ABTS cation radical upon interaction with antioxidants. The extract demonstrated a remarkable IC50 value in this assay, further confirming its classification as a strong antioxidant. The ABTS method's versatility, allowing assessment in both aqueous and lipid phases, provides a more comprehensive evaluation of the extract's antioxidant capabilities. The concordant results obtained from both DPPH and ABTS methods strengthen the evidence supporting the extract's significant antioxidant potential. The dual methodology approach enhances the reliability of the findings and provides robust validation of the extract's antioxidant properties. These

complementary methods offer a more complete understanding of the extract's potential in various physiological environments and conditions.

Analysis of MDA levels in experimental rats revealed significant variations across different treatment groups. The negative control group exhibited the highest MDA levels, indicating severe oxidative stress and extensive lipid peroxidation. In contrast, groups treated with eggplant skin ethanol extract demonstrated markedly reduced MDA levels compared to the negative control. Most notably, Treatment 6 showed MDA levels most closely resembling those of the neutral group, suggesting optimal effectiveness of the extract at this concentration. These findings provide strong evidence for the extract's ability to mitigate oxidative damage in living systems. The reduction in MDA levels demonstrates the extract's practical effectiveness. The dose-dependent response observed in the treatment groups suggests a direct relationship between extract concentration and antioxidant protection. This systematic evaluation of MDA levels provides crucial insights into the extract's biological activity and therapeutic potential.

The assessment of LPO-4HNE levels provided additional insights into the extract's protective effects against lipid peroxidation induced by oxidative stress. The negative control group showed significantly elevated LPO-4HNE levels, indicating substantial lipid damage. The treatment groups receiving eggplant skin ethanol extract exhibited progressive decreases in LPO-4HNE levels, with Treatment 6 achieving results most comparable to the neutral group. This consistent pattern of reduction in LPO-4HNE levels demonstrates the extract's effectiveness in preventing free radical-mediated lipid damage. The correlation between these results and the MDA level findings provides complementary evidence supporting the extract's protective capabilities. The comprehensive analysis of both markers provides a more complete picture of the extract's antioxidant efficacy and its potential role in preventing oxidative stress-induced cellular damage. These findings suggest promising therapeutic applications.

The study's examination of SOD activity revealed significant variations in antioxidant enzyme function across treatment groups. The negative control exhibited markedly reduced SOD activity compared to the neutral group, indicating compromised antioxidant defense mechanisms. Treatment groups receiving eggplant skin ethanol extract showed enhanced SOD activity, with Treatment 6 approaching levels similar to the neutral group. This improvement in SOD activity suggests the extract's ability to boost endogenous antioxidant defense systems. The comprehensive

analysis demonstrates the extract's significant antioxidant potential, attributed to its rich content of bioactive compounds like flavonoids and alkaloids. The consistent improvement in SOD activity across treatment groups indicates a robust and reliable antioxidant effect. These findings have important implications for the development of natural antioxidant therapies and the potential use of eggplant skin extract in medical applications.

CONCLUSION

The Neutral group demonstrated optimal physiological conditions without oxidative stress, exhibiting the lowest levels of oxidative stress markers (MDA and LPO-4HNE) and the highest SOD antioxidant activity. In stark contrast, the Negative Control group showed severe oxidative damage, characterized by significantly elevated MDA and LPO-4HNE levels alongside suppressed SOD activity. The Treatment groups displayed progressive improvement in all parameters, with Treatment 6 emerging as the most effective intervention. This optimal treatment reduced MDA and LPO-4HNE levels substantially and enhanced SOD activity, approaching levels comparable to the Neutral group. The Positive Control group showed moderate improvement compared to the Negative Control but did not achieve the same degree of effectiveness as Treatment 6. These findings convincingly demonstrate that Treatment 6 effectively mitigates oxidative stress, restores antioxidant defense mechanisms, and brings the biological system closer to normal physiological conditions.

The research design incorporated multiple complementary parameters to assess oxidative stress and antioxidant activity, strengthening the validity of the findings. The simultaneous measurement of both oxidative stress markers (MDA and LPO-4HNE) and antioxidant enzyme activity (SOD) provided a comprehensive evaluation of the treatment's effectiveness. The inclusion of both Positive and Negative Controls, alongside a Neutral group, established clear reference points for comparing treatment outcomes. The study demonstrated strong internal consistency, with Treatment 6 showing superior results across all measured parameters. The dose-dependent response observed in the Treatment groups adds reliability to the findings and helps establish optimal dosing guidelines. The research methodology followed standardized protocols for all measurements, ensuring reproducibility and reliability of results. The consistent correlation between different parameters across all treatment

groups reinforces the robustness of the experimental design and the validity of the conclusions.

Despite the significant findings, several limitations should be considered when interpreting the results. The study focused on specific oxidative stress markers and one antioxidant enzyme, potentially overlooking other relevant biochemical parameters. The research did not include long-term follow-up to assess the durability of the treatment effects or potential delayed responses. The sample size, though adequate for statistical analysis, could be expanded in future studies to enhance the generalizability of the findings. The study did not investigate the molecular mechanisms underlying the observed effects, limiting our understanding of the precise pathways involved. Additionally, the research did not examine potential interactions between the treatment and other physiological systems or medications. Future studies should address these limitations by incorporating broader parameter measurements, longer observation periods, larger sample sizes, molecular analysis, and investigation of potential systemic interactions and contraindications.

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