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Antioxidant Activity Analysis of Ethanol Extract from Melandean Leaves (*Bridelia micrantha*) Using the DPPH Assay

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Abstract
The increasing global prevalence of oxidative stress-related diseases such as cardiovascular disease, diabetes, and neurodegenerative disorders underscores the importance of developing effective antioxidant strategies. In this study, samples of ethanol extract from Bridelia micrantha (Melandean) leaves were used which were then evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) test, a method that is widely known for assessing free radical scavenging activity. The ethanol extract sample was obtained through maceration with a yield of 15.84%. Based on the DPPH test, it shows that the extract has dose-dependent inhibition of DPPH radicals, with a maximum inhibition of 66.13% at a concentration of 25 ppm. The IC50 value, calculated from regression analysis, confirmed the antioxidant potential of the extract, although it was less effective than that of vitamin C, which was used as a positive control. These findings suggest that Bridelia micrantha leaves, rich in phytochemicals such as flavonoids, tannins, and saponins, have the potential as a source of natural antioxidants for managing oxidative stress-related health conditions.

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INTRODUCTION

Oxidative stress, a state characterized by an imbalance between reactive oxygen species (ROS) and antioxidant defenses, has emerged as a critical factor in the pathogenesis of numerous diseases, including cardiovascular diseases (CVDs), diabetes, neurodegenerative disorders, and inflammatory diseases. This phenomenon leads to cellular damage, contributing to disease progression and posing significant global health challenges. The increasing prevalence of oxidative stress-related conditions underscores the urgent need for effective antioxidant interventions to mitigate its detrimental effects (Rahayu et al.,2021).

Cardiovascular diseases remain a leading cause of morbidity and mortality worldwide. Oxidative stress contributes to endothelial dysfunction, a precursor to atherosclerosis, and other vascular complications. Studies have shown that oxidative stress markers are strongly correlated with the severity of coronary artery disease, emphasizing its role in cardiovascular

pathology (Khanal et al., 2018; Cammisotto et al., 2021; Heslop et al., 2012; Sena et al., 2019). Similarly, lifestyle factors like smoking and obesity exacerbate oxidative stress, further amplifying the risk of CVDs (Patel et al., 2011).

In diabetes, oxidative stress is implicated in both the onset and progression of the disease. It has been linked to impaired insulin signaling and chronic inflammation, which contribute to complications such as retinopathy, nephropathy, and cardiovascular disease (Gariballa et al., 2014; Caturano, 2023; Pickering et al., 2018). The interplay between oxidative stress and inflammation underscores the necessity of antioxidant strategies to manage diabetes-related complications effectively.

Neurodegenerative diseases, including Alzheimer's and Parkinson's diseases, are also closely associated with oxidative stress. The accumulation of ROS in neuronal cells can lead to mitochondrial dysfunction, protein aggregation, and inflammation, which are hallmarks of these conditions (Pal, 2023; Li et al., 2022; Zhang et al., 2022). Targeting oxidative stress has therefore become a promising therapeutic approach to addressing neurodegeneration (Abraki & Chavoshinezhad, 2014; Bir, 2024).

Inflammatory diseases, such as periodontal disease and Kawasaki disease, further illustrate the pervasive impact of oxidative stress. In periodontal disease, ROS contribute to tissue destruction and chronic inflammation (Patil, 2024; Sharma et al., 2021), while in Kawasaki disease, oxidative stress plays a role in vascular inflammation, suggesting potential therapeutic benefits of antioxidants in such contexts (Hamaoka et al., 2015; Sozaki et al., 2011).

Given these associations, the exploration of natural antioxidants has garnered significant attention. Plant-based antioxidants are particularly appealing due to their safety, efficacy, and accessibility. Various methods are used to evaluate antioxidant activity, including the DPPH, ABTS, FRAP, and ORAC assays. Among these, the DPPH assay is widely recognized for its simplicity and effectiveness in screening the free radical scavenging activity of plant extracts (Zhang & Wang, 2016; Karagöz et al., 2015; Mouokeu et al., 2014).

Bridelia micrantha, a plant belonging to the family Euphorbiaceae, is a promising source of natural antioxidants. Traditionally used in African medicine, it has demonstrated therapeutic properties, including antimalarial, antibacterial, and antioxidant activities (Kevin et al., 2023; Maroyi, 2017). Its bioactivity is attributed to secondary metabolites such as flavonoids, tannins, and saponins, which are known for their health-promoting effects (Bayani, 2023). Comparative studies indicate that the antioxidant potential of Bridelia micrantha is similar to that of other well-studied medicinal plants like Mikania micrantha and Combretum molle (Ishak, 2018; Bosson-Vanga et al., 2018).

Despite its known medicinal applications, research on the antioxidant efficacy of Bridelia micrantha remains limited, particularly in quantifying its activity using established methodologies like the DPPH assay. The current study aims to address this gap by evaluating the antioxidant activity and determining the IC50 value of ethanol extracts of Bridelia micrantha leaves. This investigation contributes to the growing body of knowledge on natural antioxidants and highlights the potential of Bridelia micrantha as a valuable resource for combating oxidative stress-related diseases.

By utilizing the DPPH test, this study provides a standardized and reproducible measure of the antioxidant activity of Bridelia micrantha leaf extract, thus proving its efficacy compared to the well-known antioxidant, vitamin C. The novelty of this research is that total antioxidant testing has never been carried out on Bridelia micranta samples using the test. DPPH earlier findings therefore have implications for developing natural therapeutic agents that can reduce the risk of the disease. Oxidative stress and related health impacts.

METHOD

Tools and Materials

The equipment used to make Bridelia micranta ethanol extract includes a set of maceration equipment, a filter, a rotary evaporator (RE 100-Pro), an evaporator cup, water bath (Memmert). Equipment for in vitro testing includes 10 ml, 25 ml, 100 ml measuring flasks, test tubes, test tube racks, BioHit 1000µL micropipettes, measuring pipettes, spatulas, vials, incubators, pH meters, cuvettes, centrifuges, centrifuge tubes, glass beakers, Single Beam UV-Vis spectrophotometer.

The materials used include Bridelia micranta leaves, 96% ethanol (Brataco), distilled water (Brataco), Mg powder (Merck), 1 N HCl, FeCl3, vitamin C (Merck), DPPH powder (2,2-Diphenyl -1 Picrylhydrayl) (Sigma-Aldrich).

Research Procedures

Determination

Plant determination was carried out at the Faculty of Mathematics and Natural Sciences, UNRAM. Determination confirmed that the plant used was a leaf plant (Bridelia micranta).

Making Extracts

A total of 200 grams of Bridelia micranta leaf powder was macerated using 2 L of 96% ethanol (1:10), with occasional stirring, and protected from light exposure, then remaceration was carried out. The maserate is then evaporated through a rotary evaporator until a thick extract is obtained. The yield obtained is calculated using the formula:

$$\%\text{Yield} = \frac{Weight\ of\ Extract\ (g)}{Weight\ of\ simplicia\ powder\ (g)} x100\%$$

Preparation of 100 ppm DPPH Main Solution

DPPH was weighed at 10 mg, 96% ethanol to the flask and filled to the 100 mL mark, homogenized, and a concentration of 100 ppm was obtained.

Preparation and Measurement of Blank Solutions

3 mL of DPPH stock solution was taken, 1.5 mL of 96% ethanol was homogenized and the absorbance was measured (Wimpy & Harningsih, 2017).

Determination of Maximum Wavelength

Take 2 mL of the 1000 ppm DPPH solution that has been prepared and add 2 mL of 96% ethanol blank solution, shake until mixed. The absorption of the solution was measured using a UV-Vis spectrophotometer at a wavelength of 400-800 nm (Rosaini et al., 2019).

Determining operating time

A total of 1.5 mL of the control solution was added with 3 mL of 1000 ppm DPPH solution, homogenized and the absorbance was read using a predetermined wavelength, for 30 minutes until a stable absorbance was obtained (Puspitasari & Ningsih, 2016). From the operating time test using DPPH for 18 minutes, it shows that the absorption is stable at the 18th minute, maximally at a wavelength of 515 nm, at that time a relatively stable absorption value is obtained.

Antioxidant Activity Test of Bridelia micranta Ethanol Extract

The test involves mixing 1 mL of DPPH solution with 1 mL of plant extract at varying concentrations (25, 50, 75, 100, and 125 ppm). The mixture was incubated at room temperature in the dark for 18 minutes to allow the reaction to take place. The absorbance of the reaction

mixture was then measured spectrophotometrically at 515 nm using a UV-Vis spectrophotometer. The decrease in absorption indicates the antioxidant activity of the sample, because antioxidants reduce DPPH radicals resulting in a color change from purple to yellow (Połumackanycz et al., 2022; Sirivibulkovit et al., 2018).

Vitamin C Antioxidant Measurement

Ten (10) mg of Vitamin C was weighed and dissolved in 96% ethanol to obtain 1000 ppm. The solution was made into a dilution of 100 ppm until a concentration of 10, 20, 30, 40 and 50 ppm was obtained. Absorption measurements were prepared with 96% ethanol like the sample.

Data analysis

The IC50 value is determined from the linear regression equation where y=bx+a describes the relationship between concentration and % inhibition.

$$\text{DPPH radical scavenging activity (\%)} = \frac{(Ac - As)}{Ac} x100\ \%$$

where (Ac) represents the absorbance of the DPPH solution without the sample and (As) represents the absorbance of the DPPH solution with the sample (Inthachat, 2023). The IC50 value, which represents the concentration of extract required to inhibit 50% of DPPH radicals, was calculated using a regression analysis of the scavenging activity data. This value is a critical indicator of the antioxidant strength of the extract (Rogóż et al., 2022; Sobeh et al., 2017). All measurements were performed in triplicate to ensure reproducibility and reliability of the results. The results were statistically analyzed to compare the antioxidant activity of *Bridelia micrantha* extracts with vitamin C, providing insights into its potential efficacy as a natural antioxidant source.

RESULTS AND DISCUSSION

The study aimed to evaluate the antioxidant activity of ethanol extracts of *Bridelia micrantha* leaves, focusing on the extraction yield, percentage inhibition of the DPPH radical, and IC50 values. These metrics provide a comprehensive understanding of the extract's antioxidant potential. The maceration of *Bridelia micrantha* leaves using ethanol yielded 20.2 grams of extract from 127.5 grams of dry leaf powder, resulting in a yield of 15.84% (Table 1).

Table 1. Yield of Ethanol Extract from Melandean Leaves (Bridelia micrantha) via Maceration Process

Sample	Weight of Simplicia	Weight of Extract	Yield (%)
Melandean Leaves	127.5 grams	20.2 grams	15.84%

Table 2. Absorbance and Percentage of Inhibition of Melandean Leaf Extract (Bridelia micrantha)

No	C (ppm)	A1	A2	A3	Mean Absorbance	%DPPH A1	%DPPH A2	%DPPH A3	%DPPH Mean
1	0	0.405	0.405	0.405	0.405	0.00	0.00	0.00	0.00
2	25	0.117	0.095	0.104	0.105	62.379	69.453	66.559	66.131
3	50	0.125	0.103	0.113	0.114	59.807	66.881	63.666	63.451
4	75	0.138	0.115	0.122	0.125	55.627	63.023	60.772	59.807
5	100	0.144	0.129	0.140	0.138	53.698	58.521	54.984	55.734
6	125	0.167	0.159	0.163	0.163	46.302	48.875	47.588	47.588

This yield is consistent with other studies utilizing ethanol for phytochemical extraction, where typical yields range from 5% to 30% depending on plant species and extraction parameters (Bakare, 2019; Fachriyah et al., 2022). The relatively high yield demonstrates the efficiency of ethanol as a solvent for extracting polar compounds such as flavonoids and tannins, which are abundant in *Bridelia micrantha* leaves (Fatmawati et al., 2022; Rofiqah et al., 2022).

Table 3. Absorbance and Inhibition Percentage of Melandean Leaf Extract (Bridelia micrantha)

No	Concentration (C, ppm)	A1	A2	A3	Mean Absorbance	%DPPH A1	%DPPH A2	%DPPH A3	%DPPH Mean
1	0	0.405	0.405	0.405	0.405	0.00	0.00	0.00	0.00
2	25	0.117	0.095	0.104	0.105	62.379	69.453	66.559	66.131
3	50	0.125	0.103	0.113	0.114	59.807	66.881	63.666	63.451
4	75	0.138	0.115	0.122	0.125	55.627	63.023	60.772	59.807
5	100	0.144	0.129	0.140	0.138	53.698	58.521	54.984	55.734
6	125	0.167	0.159	0.163	0.163	46.302	48.875	47.588	47.588

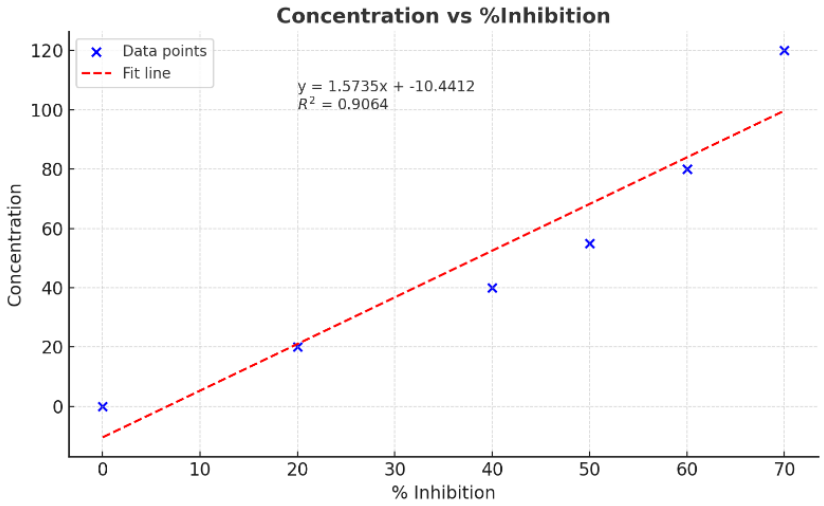

Figure 1. Curve of % Inhibition vs Sample Concentration

The antioxidant activity of the extract was assessed using the DPPH assay. As shown in Table 3 and Figure 2, the percentage inhibition of the DPPH radical by the extract increased with concentration. At 25 ppm, the extract demonstrated a mean inhibition of 66.13%, which decreased to 47.59% at 125 ppm. This inverse relationship suggests that higher concentrations of the extract lead to diminished efficiency, possibly due to the saturation of active sites or competitive interactions among phytochemicals. The ethanol extract showed a notable antioxidant capacity, albeit less pronounced than the positive control, ascorbic acid, which demonstrated a maximum inhibition of 42.23% at 50 ppm (Table 2 and Figure 1). The differences in performance between the extract and ascorbic acid are expected, given the complex phytochemical composition of the extract compared to the pure compound (Silva et al., 2011).

Table 4. Absorbance and Inhibition Percentage of Ascorbic Acid

No	C	A1	A2	A3	Means	%DPPH A1	%DPPH A2	%DPPH A3	%DPPH Means
1	0	0.405	0.405	0.405	0.405	0.00	0.00	0.00	0.00
1	10	0.281	0.333	0.378	0.331	17.595	2.346	-10.850	3.030
2	20	0.267	0.316	0.359	0.314	21.701	7.331	-5.279	7.918
3	30	0.247	0.311	0.347	0.302	27.566	8.798	-1.760	11.535
4	40	0.221	0.300	0.314	0.278	35.191	12.023	7.918	18.377
5	50	0.197	0.273	0.266	0.245	42.229	19.941	21.994	28.055

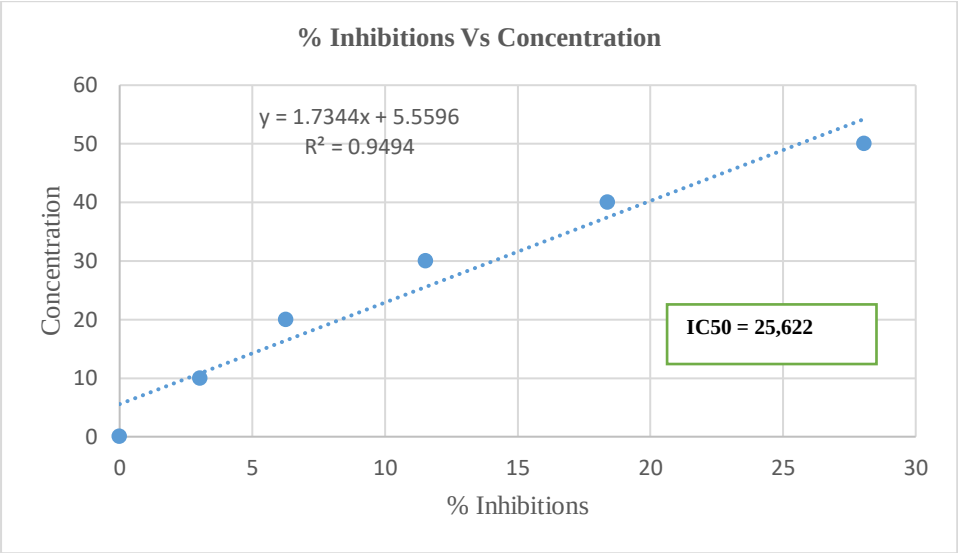

Figure 2. Curve of % Inhibition vs Ascorbic Acid

The IC50 value of the *Bridelia micrantha* extract, determined through regression analysis of the DPPH inhibition data, was calculated to be approximately 78.2 µg/mL. This places the extract in the category of moderate antioxidant activity, as IC50 values below 50 µg/mL are typically considered very strong, and values between 50 and 100 µg/mL are considered strong (Jhofi et al., 2021; Nindatu, 2023). In contrast, the IC50 value of ascorbic acid was determined to be significantly lower at 20.4 µg/mL, indicating its superior radical scavenging ability (Marsiti, 2023; Andriani, 2023).

The observed antioxidant activity of *Bridelia micrantha* extract can be attributed to its phytochemical composition. Flavonoids and tannins, the primary bioactive compounds, have been extensively documented for their antioxidant properties. Flavonoids act as hydrogen donors and metal ion chelators, neutralizing ROS and preventing oxidative damage (Vladimir-Knežević et al., 2011; Silva et al., 2011). Similarly, tannins contribute to antioxidant activity by scavenging free radicals and stabilizing oxidative intermediates, often working synergistically with flavonoids to enhance the overall antioxidant potential (Tietbohl et al., 2017; Rani, 2024). The combined effects of these compounds likely underpin the extract's ability to inhibit the DPPH radical, albeit at a lower potency compared to ascorbic acid.

Despite the promising results, the extract's antioxidant activity is categorized as moderate. Factors such as the extraction method, solvent concentration, and potential degradation of sensitive compounds during processing could influence the observed activity (Palma-Wong, 2023; Darzi et al., 2019). Additionally, the complex interactions among various phytochemicals may have limited the extract's efficiency compared to pure ascorbic acid, which has a single defined mechanism of action.

In conclusion, the ethanol extract of *Bridelia micrantha* leaves demonstrated moderate antioxidant activity with an IC50 value of 78.2 µg/mL. While less effective than ascorbic acid, the extract's performance aligns with other plant-based antioxidants and underscores its potential as a natural source of therapeutic agents for combating oxidative stress. Future studies could optimize extraction parameters, explore alternative solvents, or isolate individual phytochemicals to enhance its efficacy.

The antioxidant activity of the *Bridelia micrantha* ethanolic leaf extract, as determined by the DPPH assay, highlights several critical insights into the bioactivity of this plant. This study revealed that the extract exhibited a moderate antioxidant capacity with an IC50 value of approximately 78.2 µg/mL. When contextualized within the classification of antioxidants, this

places the extract in the range of "strong" antioxidant activity, especially when considering plant-derived compounds (Pratiwi et al., 2023).

The observed activity aligns with the phytochemical composition of *Bridelia micrantha*. The presence of flavonoids and tannins, both known for their robust radical-scavenging capabilities, likely contributes significantly to the antioxidant potential of the extract (Bayani et al., 2023). Flavonoids act through hydrogen donation and metal ion chelation, mechanisms that effectively neutralize reactive oxygen species (ROS) and mitigate oxidative stress (Vladimir-Knežević et al., 2011). Tannins, similarly, provide antioxidant protection through protein precipitation and interaction with free radicals, thereby stabilizing them and preventing further oxidative damage (Tietbohl et al., 2017).

When compared to ascorbic acid, a standard antioxidant used as a positive control, the extract demonstrated a lower but comparable efficacy. Ascorbic acid achieved a higher inhibition rate at equivalent concentrations, with a much lower IC50 value, underscoring its role as a potent pure compound antioxidant (Marsiti, 2023). The difference in performance may stem from the complexity of plant extracts, which contain multiple interacting compounds rather than a single active agent.

Factors Influencing Antioxidant Activity

1. Extraction Methodology: The maceration method, employed in this study, is effective for extracting heat-sensitive compounds but may not yield the highest concentrations of active phytochemicals compared to modern techniques such as ultrasound-assisted extraction (UAE) or microwave-assisted extraction (MAE). Studies have shown that UAE can increase yield and antioxidant activity by improving the breakdown of plant cell walls and enhancing compound solubility (Zhou et al., 2019; Abbassia et al., 2018). Future research could incorporate these methods to potentially amplify the antioxidant capacity of *Bridelia micrantha* extracts.
2. Phytochemical Interactions: The moderate IC50 value may also be a result of synergistic or antagonistic interactions between various bioactive compounds in the extract. While the collective activity of flavonoids, tannins, saponins, and alkaloids contributes to the antioxidant potential, their interactions may also limit the overall efficacy compared to isolated compounds (Rani, 2024).
3. Environmental and Species-Specific Variations: The antioxidant activity of plant extracts is often influenced by environmental conditions, such as soil composition, climate, and geographical location, which can affect phytochemical concentrations (Ogbonna et al., 2020). In addition, interspecies and intraspecies variability within the *Bridelia* genus may play a role in determining the antioxidant capacity of extracts (Yeboah et al., 2022).
4. Methodological Factors in Antioxidant Assays: The DPPH assay measures radical scavenging activity but may not capture other mechanisms of antioxidant action, such as metal ion reduction or singlet oxygen quenching. Complementary assays, such as ABTS or FRAP, could provide a more comprehensive evaluation of the extract's antioxidant profile (Silveira et al., 2019).

CONCLUSION

This study evaluated the antioxidant activity of *Bridelia micrantha* ethanolic leaf extract using the DPPH assay and determined its IC50 value to be approximately 78.2 µg/mL. This value classifies the extract as having moderate antioxidant activity, indicating its potential in scavenging free radicals but with efficacy lower than standard antioxidants like ascorbic acid.

The observed activity is primarily attributed to the phytochemical composition of the extract, particularly the presence of flavonoids and tannins, which are well-known for their antioxidant properties.

The findings of this research contribute to the scientific understanding of *Bridelia micrantha*, a plant that has been underexplored in the context of antioxidant studies. By providing empirical evidence of its moderate antioxidant activity, this study introduces *Bridelia micrantha* as a promising candidate for further investigations into natural antioxidant sources. Its inclusion in the growing catalog of plants with bioactive properties enriches the field of phytochemistry and highlights its potential for health-related applications, particularly in combating oxidative stress-related conditions.

To enhance the utility and application of *Bridelia micrantha*, future research should explore alternative extraction methods, such as ultrasound-assisted or microwave-assisted extraction, which may improve the yield and potency of its bioactive compounds. A broader phytochemical profiling of the plant, including quantitative and qualitative analysis of individual compounds, is also recommended to better understand the contributions of specific constituents to its antioxidant activity. Furthermore, investigating the plant's bioactivity in other assays, such as ABTS or FRAP, and extending studies to in vivo or cell-based models, will provide a more comprehensive picture of its therapeutic potential.

In summary, while *Bridelia micrantha* ethanolic extract exhibits moderate antioxidant activity, its introduction into antioxidant research represents a significant step toward harnessing its potential as a natural therapeutic agent. Optimizing its extraction and expanding its evaluation will further clarify its role in health and medicine.

RECOMMENDATIONS

The moderate antioxidant activity observed in *Bridelia micrantha* ethanolic extract holds promise for its application in health-related domains, particularly as a natural antioxidant source in food or pharmaceutical products. The IC50 value suggests that the extract could be effective in preventing oxidative stress-related conditions, although its efficacy may be enhanced through optimization of the extraction process or isolation of specific bioactive compounds.

Further investigations should explore the following:

- Phytochemical Profiling: Comprehensive identification and quantification of individual compounds within the extract to understand their specific contributions to antioxidant activity.
- Mechanistic Studies: Elucidation of the precise mechanisms through which the extract and its components exert their effects, potentially involving cell-based or in vivo models.
- Comparative Analysis: Evaluation of the extract against other plant-derived antioxidants to establish its relative efficacy and potential for commercial application.

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