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Phytochemical Screening and Antioxidant Activity of *Spondias pinnata* (L.f.) Kurz Nano Extract

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ABSTRACT:

Background: S*pondias pinnata* (L.f) Kurz, commonly known as cemcem, is a medicinal plant recognized for its numerous health benefits. All parts of the cemcem plant are valuable due to their potential medicinal properties, which exhibit various biological activities, including antibacterial and antioxidant effects. The traditional extract has been utilized, which comes with certain limitations. To enhance the effectiveness of these compounds, this study employed a nano extract, which refers to an extraction method that produces particles on the nanometer scale. This approach aims to improve absorption, increase solubility, and reduce the required dosage. **Objective:** The study aimed to identify the phytochemical compounds in cemcem leaf nano extract using 96% ethanol and to determine its antioxidant activity. **Methods:** This research is a descriptive study. he phytochemical profile, including alkaloids, flavonoids, tannins, saponins, and steroids, was determined through qualitative tests. Antioxidant activity was determined using the DPPH method. **Results:** The phytochemical test showed that the nano extract of cemcem leaves contains flavonoids, phenols, tannins, and saponins. The antioxidant activity of the sample was measured and expressed with an IC50 value of 200.88 ppm. These results indicate that the antioxidant activity of nano extracts from cemcem leaves is significantly higher than that of conventional extracts. **Conclusion:** Based on the results, it can be concluded that the nano extract from cemcem leaves has the potential to be developed as a natural antioxidant.

Keywords: Antioxidant; Cemcem; Nano_extract; Phytochemicals; *Spondias pinnata* (L.f.) Kurz)

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INTRODUCTION

Indonesia is one of the biodiversity countries in the world. Indonesia has approximately 31,750 plant species, of which around 15,000 have the potential to be medicinal plants. However, only about 7,000 species are currently utilized as raw materials for medicine. The Indonesian people have a long history of using various plants to produce medicinal products that help maintain health.

One of the plants that is widely used as traditional medicine is cemcem (*Spondias pinnata* (L.f.) Kurz) (Cahyaningsih et al., 2021; Habibah, Bekti, et al., 2024). Cemcem leaves are processed as raw material for a traditional Balinese drink known as *loloh cemcem*, which is traditionally used for treating diabetes, urolithiasis, and stomach disorders (Habibah, Bekti, et al., 2024; Laksemi, 2019; Sujarwo et al., 2017; Sutana, 2020; Trisnawati et al., 2016).

Cemcem contains several bioactive compounds, including steroids, saponins, flavonoids, tannins, vitamin C, organic acids, and terpenoids, many of which possess antioxidant properties (Bekti et al., 2022; Dharmawati et al., 2022; Habibah, Bekti, et al., 2024; Laksemi, 2019; Sujarwo et al., 2017). Antioxidants are compounds that can neutralize or scavenge free radicals, thereby helping to prevent degenerative diseases such as cardiovascular disorders and cancer. These compounds are crucial for the body as they protect against damage to normal cells, proteins, and lipids caused by free radicals. Free radicals are unstable atoms or molecules that have one or more unpaired electrons. They can arise from various sources in our environment, including cigarette smoke, UV radiation, vehicle emissions, and fried foods. Prolonged exposure to free radicals can lead to multiple health issues, including premature aging, heart disease, cataracts, cancer, and other degenerative disorders (Ardani et al., 2023; Sharifi-Rad et al., 2020; Sridevi et al., 2018; Tatiana & Ria, 2020).

Antioxidants are essential for preventing diseases in the body. Although humans naturally produce endogenous antioxidants, the quantity is often insufficient to neutralize

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excessive free radicals. This shortfall necessitates the intake of additional exogenous antioxidants. Antioxidants can be classified as synthetic or natural. However, the use of synthetic antioxidants is restricted by government regulations due to concerns about toxicity that may arise from their excessive consumption. As a result, there is a need for safe alternative antioxidants, with plants emerging as one of the most promising sources of natural antioxidants (Ardani et al., 2023; Colica et al., 2018; Poljsak et al., 2021; Sharifi-Rad et al., 2020).

Previous studies on cemcem have generally been carried out using crude extracts. However, the use of plant extracts has several limitations, such as low bioavailability of active compounds, instability of bioactive molecules, and relatively ineffective antibacterial activity against pathogens (Bekti et al., 2022; Dharmawati et al., 2022; Habibah, Bekti, et al., 2024; Laksemi, 2019; Putu Sulistiawati Dewi, 2023; Sujarwo et al., 2017; Trisnawati et al., 2016). To overcome these limitations, one potential solution is the development of nano-extracts. A nano-extract is a natural extract whose particle size has been reduced to the nano-meter scale, typically 10–1000 nm. The advantages of nano-extracts lie in their enhanced delivery system for active compounds. Particles at the nanometer scale possess unique physical properties compared to larger particles, leading to several benefits. These benefits include improved solubility of bioactive compounds, reduced therapeutic doses, and enhanced absorption. With smaller particle sizes, nano-extracts facilitate better absorption efficiency and increase the biological activity of the active compounds. Additionally, this means that less raw material is needed in formulations since lower doses are sufficient to achieve the desired therapeutic effect. (Bilia et al., 2018; Ghosh et al., 2025; Jadhav & Jadhav, 2022; Sa'diah et al., 2023; Srivastava, 2025). One commonly used method for synthesizing nano-extracts is the ionic gelation method. It is favored for its simplicity and because it avoids the need for heating, high agitation, and hazardous organic solvents. This method involves using chitosan polymer and sodium tripolyphosphate (Na-TPP) as a cross-linking agent. The combination of these two materials results in the formation of particles with strong mechanical bonds (Abdassah, 2017; Windy et al., 2022).

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However, the potential of *Spondias pinnata* (L.f.) Kurz. nano-extracts as antioxidant agents have not been widely studied. Therefore, this research aims to identify the phytochemical profile and determine the antioxidant activity using UV-Vis spectrophotometry to develop natural antioxidant agents.

METHOD

This study is a descriptive study that aims to describe the phytochemical profile and antioxidant activity of nano extracts from cemcem leaves. The sample in this research is nano extract of cemcem leaves (*Spondias pinnata* (L.f.) Kurz). The nano-extract was obtained through a maceration process and evaporation, followed by synthesis of the nano extract using an ionic gelation method with Na-TPP and chitosan. Cemcem leaves that met the research criteria were obtained from Penglipuran Village, Bangli, Bali, Indonesia. Phytochemical screening was carried out using qualitative analysis to identify the secondary metabolite compounds contained in the sample, such as alkaloids, flavonoids, tannins, saponins, and steroids. Antioxidant activity was determined with DPPH method by using UV-Vis spectrophotometer. The tests were conducted at the Laboratory of P4TO, Bali Province and Chemical-Toxicological Laboratory, Medical Laboratory Department, Poltekkes Kemenkes Denpasar, in January-March 2025.

Furthermore, the data obtained were recorded, processed, presented in a tabulation, and narrated and discussed in accordance with the theory and related literature. This research has been reviewed and obtained Ethical Approval from the Health Research Ethics Commission of the Denpasar Health Polytechnic Number: DP.04.02/F.XXXII.25/392/2025.

Preparation of Nano Extract of Cemcem Leaves (*Spondias Pinnata* (L.F.) Kurz)

The selected leaves were washed thoroughly with tap water, then the leaves were dried using an oven at a temperature of 40 ± 1°C until completely dry. After drying, the leaves were sorted to separate them from the stems and other parts that were involved in

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drying. Leaves then crushed using a blender and sieved to obtain simplisia powder. Next, the simplisia powder was stored in a closed and airtight jar at room temperature. The obtained *Pluchea indica L.* leaves powder was then used in the extraction process. The extraction process was performed by weighing 400 g of dried leaves, which are then dissolved in 96% ethanol at a ratio of 1:5. The obtained extract was filtered with filter paper. The obtained filtrate was concentrated in the rotary evaporator vacuum at 30°C (Habibah, Ardani, et al., 2023; Habibah, Bekti, et al., 2024). The extract yield obtained based on the mass of simplisia powder was 30.57%.

A 1 gram of extract was dissolved in 35 mL of ethanol and mix with 15 mL of distilled water. Then, 50 mL of a 0.2% chitosan solution prepared in 1% glacial acetic acid was added. Add 50 mL of 0.2% sodium tripolyphosphate (Na-TPP) while stirring with a magnetic stirrer at 400 RPM for 20 minutes. Afterward, centrifuge the mixture at 3000 RPM for 15 minutes. Finally, the nanoextract was characterized by measuring the percentage transmittance using spectrophotometry at a wavelength of 650 nm (Ramadhani, 2020).

Phytochemical Screening of Nano Extract of Cemcem Leaves (*Spondias Pinnata* (L.F.) Kurz)

Alkaloids A volume of 1 mL of the sample was added to an ammonia-chloroform solution, and the mixture was shaken for one minute before being filtered. After filtration, 5 mL of sulfuric acid (H_2SO_4) was added, and the mixture was shaken again. It was then allowed to settle, and the aqueous phase was separated for further testing. Next, Mayer's reagent was introduced. For the **flavonoid** test, 5 mL of the sample was placed in a glass beaker, mixed with 10 mL of ethyl alcohol, and heated to boiling. After filtering the mixture, 0.5 mL of the filtrate was added to 1 mL of dilute ammonia solution, and any changes in the sample were observed. For the **tannin** test, 1.6 mL of the sample was added to an $FeCl_3$ solution, and color changes were noted. To test for the presence of phenols, 2 mL of the sample was transferred to a conical flask using a pipette, followed by a few drops of $FeCl_3$. For the **saponin** test, 10 mL of the sample was mixed with 5 mL of distilled water and vigorously shaken until foam formed. Three drops of olive oil were then added to the

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mixture, which was shaken again, and observations were made regarding the formation of an emulsion (Habibah, Nugroho, et al., 2023).

Antioxidant Activity of Nano Extract of Cemcem Leaves (*Spondias Pinnata* (L.F.) Kurz)

A series concentration of nano extract of cemcem leaves (*Spondias Pinnata* (L.F.) Kurz) 30, 60, 90, 120 and 150ppm was prepared. 2 mL sample was added to 2 mL of 0.1mM DPPH solution in methanol. The mixed solution was then vortexed and incubated for 30 minutes at room temperature. The absorbance of the solution was then measured at 516 nm. Antioxidant activity was expressed by the percentage of inhibition (IC_{50}), which was calculated based on = [(AC-AS)/AC] x 100%, where AC = control absorbance, and AS = sample absorbance (Ardani et al., 2023; Habibah, Nugroho, et al., 2023).

The obtained results were adjusted to the classification categories based on IC_{50} values, presented in tables, and described narratively.

Table 1. Range of IC_{50} Values

IC_{50} Value (ppm)	**Category**
<50	Very Strong
50-100	Strong
100-150	Moderate
150-200	Weak
>200	Very Weak

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RESULTS AND DISCUSSION

A. Results

1. Nano Extract of Cemcem Leaves (*Spondias Pinnata* (L.F.) Kurz)

In this study, the sample used was the nano extract of cemcem leaves. The leaves were obtained from Penglipuran Village, Bangli, Bali, Indonesia. The selected leaves had to meet specific criteria: they needed to be intact, fresh, and green, without any signs of rot, pest damage, or disease. Additionally, the leaves should not be dry or have holes, and only those taken from the tip up to the fifth petiole were included. The moisture content and yield were tested, as detailed in Table 2.

Table 2. Results of Moisture Content and Yield Test

No.	Test	Result
1.	Moisture Content	4.384%
2.	Yield	30.57%

In the drying process of cemcem leaves, the oven temperature was set to 45 °C. Previous studies recommend a drying temperature ranging from 20 °C to 90 °C. The moisture content measured after testing was 4.384%. This water content in cemcem simplisia leaves meets the established standard, as literature indicates that the moisture content in extracts should not exceed 10% to prevent rapid fungal growth (Fauzi et al., 2022; Wandira et al., 2023; Widayanti et al., 2023).

In this study, 96% ethanol was used as the solvent for the extraction process. Ethanol was chosen due to its polarity, common usage, and high extraction efficiency. It can dissolve compounds with varying levels of polarity, ranging from nonpolar to polar. The 96% concentration was selected because it is more effective at penetrating cell walls than lower concentrations,

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resulting in a more concentrated extract. The extraction process continued until the maximum amounts of active compounds was obtained. Reprecipitation was performed to enhance extraction efficiency. This method operates on the principle of using alternating solvents to dissolve secondary metabolites found in herbal materials, depending on their solubility characteristics. Following this, evaporation was conducted at 50 °C until a thick extract was achieved. The evaporation process's temperature and duration significantly influence the water content; as the temperature increases and the evaporation time extends, the remaining water content decreases. In this study, the extract yield was 30.57%. Extract yield calculations are conducted to determine the ratio of the extract amount obtained to the crude material's initial weight. Additionally, the extract yield can indicate the quantity of bioactive compounds present in the extracted material. A higher extract yield suggests that more bioactive compounds have been successfully extracted (Fatwami & Royani, 2023; Habibah, Ayu Aprilia, et al., 2024; Martini et al., 2020; Widayanti et al., 2023).

The nano extract was produced using the ionic gelation method. This method offers several advantages, including its simplicity, the absence of organic solvents, and ease of control, which allows for the formation of nanoparticles through ionic gelation. Ionic gelation involves the crosslinking of polyelectrolytes with multivalent counter-ions. For the preparation of the nano extract, chitosan and sodium tripolyphosphate (Na-TPP) were used as encapsulating and crosslinking agents. Chitosan is a polysaccharide derived from the deacetylation of chitin, a substance found in the shells of crustaceans such as shrimp and crabs. Chitosan was chosen for its bioactive, biocompatible, and biodegradable properties. However, chitosan also has limitations, such as rapid water absorption, which can cause swelling and reduce the efficiency of drug delivery and release. To address this issue, NaTPP was added as a crosslinking agent to decrease swelling and enhance biocompatibility. NaTPP is a non-toxic, polyanionic compound that interacts electrostatically with chitosan, forming ionic crosslinks. Due to its quick gel-forming ability, NaTPP is commonly used in the preparation of chitosan beads and microspheres (Abdassah, 2017; Guge et al., 2024; Windy et al., 2022).

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The nano extract was obtained through an encapsulation process, and its transmittance was measured using a UV-Vis spectrophotometer at a wavelength of 650 nm. The results are presented in Table 3. The average transmittance was found to be 93.04%, indicating that the formation of the cemcem nano extract was relatively successful. A quality nano extract should be clear and have a transmittance value greater than 90%. A value close to 100 suggests that the particle sizes are smaller, which leads to larger surface areas and enhances absorbance readings. Smaller particles also promote Brownian motion, preventing sedimentation and ensuring that the solution remains clear. Based on Table 3, the transmittance of the nano extract sample was 93.04%, which meets the requirement of nano extract samples, namely in the range of 90–100%(Abdassah, 2017; Ramadhani, 2020; Srivastava, 2025; Windy et al., 2022).

Table 3. Transmittance Result of Nano Extract of Cemcem Leaves

No.	Replication	Visual Apperance	Transmittance
1.	1	Clear Solution	93,07%
2.	2	Clear Solution	93,03%
3.	3	Clear Solution	93,03%
Average			93,04% $\pm$ 0.018

2. Phytochemical Screening of Nano Extract of Cemcem Leaves (*Spondias Pinnata* (L.F.) Kurz)

Phytochemical screening of the 96% ethanol nano extract of cemcem leaves was carried out qualitatively, and the results are presented in Table 4.

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Table 4. Phytochemical Screening Results of Nano Extract of Cemcem Leaves

No.	Test Parameter	Result	Observed Change
1.	Alkaloid (Mayer)	-/Negative	No white precipate
2.	Alkaloid (Wagner)	-/Negative	No reddish-brown precipate
3.	Flavonoid	+/Positive	Color change to orange
4.	Tannin	+/Positive	Color change to greenish black
5.	Phenol	+/Positive	Color change to bluish black
6.	Saponin	+/Positive	Foam formation

Phytochemical screening of the 96% ethanol nano extract from cemcem leaves revealed positive results for flavonoids, tannins, phenols, and saponins, while alkaloids yielded negative results when tested with Mayer's and Wagner's reagents.

a. Flavonoids: The test reaction exhibited an orange color change. When magnesium powder and 10% hydrochloric acid (HCl) were added, the solution shifted from green to orange, indicating a positive result. Magnesium powder facilitates carbonyl binding in flavonoids, while HCl acidifies the solution, forming red-orange flavilium salts that serve as indicators of flavonoids (Ardani et al., 2023; Habibah, Nugroho, et al., 2023; Habibah & Ratih, 2023; Harborne, 1984; Nurjannah et al., 2022).

b. Tannins: This test produced a dark green color change, indicating the formation of complexes between tannins and iron (III) chloride ($FeCl_3$). Tannins are polar due to their multiple hydroxyl (-OH) groups, allowing them to dissolve well in polar solvents like methanol (Ardani et al., 2023; Habibah, Nugroho, et al., 2023; Halimu et al., 2017; Harborne, 1984).

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c. Phenols: The test resulted in a blue-black color, confirming the presence of phenolic compounds. Phenols react with $FeCl_3$ to form colored complexes (green, purple, red, blue, brown, or black) based on the number and position of hydroxyl groups (Habibah, Nugroho, et al., 2023; Harborne, 1984; Syamsudin et al., 2022).
d. Saponins: The presence of foam indicated the existence of saponins. These complex glycosides are water-soluble surface-active compounds that hydrolyze into glycone and aglycone. Known as natural surfactants, saponins can be effectively extracted from plants using methods such as maceration or Soxhlet extraction (Habibah, Nugroho, et al., 2023; Harborne, 1984).
e. Alkaloids: The absence of white precipitate in Mayer's reagent and no reddish-brown precipitate in Wagner's reagent indicated negative results. In positive tests, these precipitates form due to interactions with nitrogen ions, but their absence confirms that alkaloids are not present (Habibah, Nugroho, et al., 2023; Harborne, 1984; Putri & Lubis, 2022).

3. Antioxidant Activity of Nano Extract of Cemcem Leaves (*Spondias Pinnata* (L.F.) Kurz)

The antioxidant activity of the 96% ethanol extract of cemcem leaves was tested using a UV-Vis spectrophotometer employing the DPPH method. This method measures the antioxidant capacity of the sample based on its ability to reduce DPPH free radicals. The purple DPPH radical changes color to pale yellow after reacting with antioxidant compounds. This color change can be observed through a decrease in absorbance values at a specific wavelength (Ardani et al., 2023; Rahmawati et al., 2016).

Before conducting the antioxidant activity test, the initial step was to determine the maximum wavelength (λmax) to identify the wavelength with the highest absorbance. Determination of the maximum wavelength (λmax) of 0.1 mM DPPH in the range of 450–650 nm using a UV-Vis spectrophotometer. Measurements revealed that the maximum wavelength was 516 nm, with an absorbance value of 0.4366. This wavelength served as a reference for measuring the absorbance of the samples during the antioxidant activity test. Measuring at the maximum wavelength increases sensitivity and minimizes errors, as changes in absorbance per concentration unit are most significant at this specific wavelength. The determination of the maximum

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wavelength using a UV-Vis spectrophotometer confirmed that the maximum absorbance of DPPH occurred at 516 nm (Masykuroh & Abna, 2022).

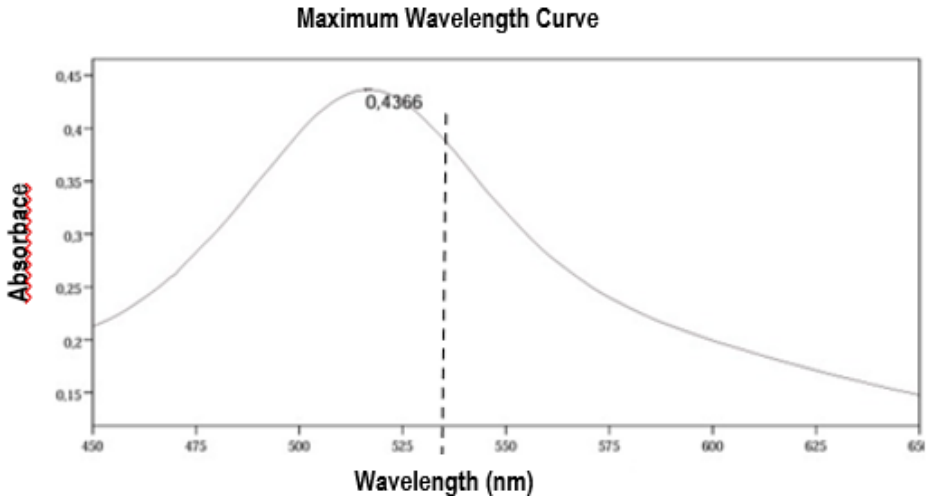

Figure 1. Maximum Wavelength Curve

The antioxidant activity test of the nano ethanol extract of cemcem leaves is presented in the Table 5 and Figure 2. The absorbance and inhibition percentage of a series concentration of sample at a concentration of 0, 30, 60, 90, 120, and 150 ppm are presented in Table 5.

Table 5. The absorbance and inhibition percentage of Nano Extract of Cemcem Leaves

No	Sample Concentration (ppm)	Average Absorbance	% Inhibition
1.	0	0,4180 ± 0.000367	0
2.	30	0,4071 ± 0.000606	2.607
3.	60	0,3661 ± 0.000230	12.416
4.	90	0,3329 ± 0.000414	20.358
5.	120	0,3036 ± 0.000327	27.368
6.	150	0,2764 ± 0.000432	33.875

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Based on the percentage of inhibition, the linear regression equation between the concentration series and the percentage of inhibition can be observed in the figure below:

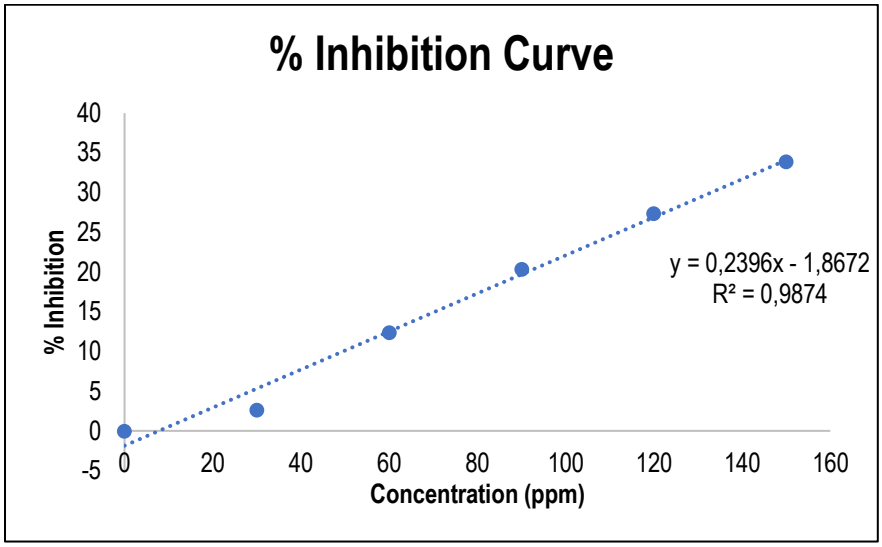

Figure 2. Results percentage of inhibition

Antioxidant activity testing was performed on a series of concentrations: 0, 30, 60, 90, 120, and 150 ppm. After obtaining the % inhibition, a curve was generated between the concentration (ppm) and inhibition levels of the cemcem leaves nano extract, resulting in an R^2 value of 0.9874. This value indicates that the curve exhibited linear characteristics. The closer the regression coefficient is to 1, the stronger and better the relationship between concentration and inhibition level (Ardani et al., 2023; Cvetanović et al., 2020; Martiningsih et al., 2016).

The antioxidant activity of the cemcem leaves nano extract was expressed as the IC50 value. The IC50 was calculated based on the obtained regression equation. The IC50 value of the nano extract samples in this study was 200.88 ppm, while the cemcem leaf extract showed an IC50 value of 413.12 ppm. These results prove that the antioxidant activity of nano extracts from cemcem leaves is greater than that of the extract. The results of the antioxidant activity test of cemcem leaf extract showed an IC50 value of 413.12 ppm. The higher the IC50 value of a

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substance, the lower its antioxidant activity. The results of the antioxidant activity test of the nano sample extract, indicated by an IC50 value of 200.18 ppm, show that at this concentration, the nano extract sample can inhibit 50% of DPPH free radicals. The low activity may be attributed to the limited composition of bioactive compounds in the extract, particularly the absence of alkaloids, which are known to contribute to free radical scavenging mechanisms (Ardani et al., 2023; Hikmawanti et al., 2021; Powthong & Suntornthiticharoen, 2023).

Although the nano formulation process achieved an efficiency of >90% and theoretically could enhance the solubility and bioavailability of active compounds, the absence of alkaloid bioactive compounds and the suboptimal levels of flavonoids, phenols, or tannins likely remain the main limiting factors. Environmental factors such as climate and soil moisture can also influence the low antioxidant activity results. Air humidity helps prevent cuticular dehydration and reduces water stress in plants. High humidity levels may lead to decreased total flavonoid content in plants. Environmental conditions, including stress factors, play an essential role in synthesizing secondary metabolites. One such stressor is soil moisture. When soil moisture is high, flavonoid levels and antioxidant activity tend to increase, whereas low soil moisture generally results in reduced flavonoid content and antioxidant capacity (Martiningsih et al., 2016). Nevertheless, these findings still indicate the potential antioxidant activity.

CONCLUSION

This study proves that nano extracts of cemcem leaves (Spondias pinnata (L.f.) Kurz) contain various phytochemical compounds, including flavonoids, tannins, phenols, and saponins. An IC50 value of 200.88 ppm indicates the antioxidant activity of these nano extracts. The findings also reveal that the nano extracts exhibit greater antioxidant activity than the original extracts. Based on these results, it can be concluded that nano extracts from cemcem leaves have significant potential for development as natural antioxidant agents.

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Conflict of Interest

During the research, there is no conflict of interest related to this research and publication.

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