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Antioxidant Activity of Nanoparticle Modified from Sembung Leaf (*Blumea balsamifera* L.) Extract

Aktivitas Antioksidan Nanopartikel dari Ekstrak Daun Sembung (Blumea balsamifera L.)

Iza Ayu Saufani[1], Azimatur Rahmi[2,3*], Tika Afriani[3], Widi Urdatullah Zikirzi[3]

[1]Program Studi Gizi, Fakultas Teknik, Universitas Negeri Medan, Jl. William Iskandar Ps.V, Deli Serdang, Sumatera Utara 20221, INDONESIA
[2]Program Studi Kimia, Fakultas MIPA Militer, Universitas Pertahanan, Kawasan IPSC Sentul, Bogor, Jawa Barat 16810, INDONESIA
[3]Program Studi Farmasi, Fakultas Ilmu Kesehatan, Universitas Mohammad Natsir Bukittinggi, Jl Tan Malaka Bukit Cangang Kayu Ramang, Bukittinggi, Sumatera Barat 26138, INDONESIA

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Correspondent:
Azimatur Rahmi
azimatur.rahmi046@gmail.com

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ABSTRACT

Background: Dietary antioxidants are essential to prevent free radical-induced cell damage and maintain the body's physiological functions. Sembung leaves are rich in polyphenols, which are known to have substantial antioxidant activities. The antioxidant efficacy of Sembung leaves can be enhanced by nanoparticle size.
Objectives: To examine the enhancement of antioxidant activity of Sembung leaf (*Blumea balsamifera* L.) extract modified into nanoparticles.
Methods: The extract used was an ethyl acetate fraction extract. The fresh leaves of Sembung ware macerated in 96% ethanol for 24 hours. Ethanol, n-hexan, and ethyl acetate solvents were used to fractionate the ethanolic extract. The extract of the ethyl acetate fraction was modified into nanoparticles through the ionic gelation method. The nanoparticles comprised three formulations, F1 (0.25% chitosan and 10 ml NaTPP solution), F2 (0.50% chitosan and 20 ml NaTPP solution), and F3 (0.75% chitosan and 30 ml NaTPP solution). The antioxidant activity was assessed by lipid peroxidation using ferric thiocyanate (FTC) followed by the thiobarbiturat (TBA) method. A Spectrophotometer UV-Vis was used to analyse the % inhibition values of the samples.
Results: The antioxidant activity assay on the sample of ethyl acetate fraction using the FTC method showed 31.25% inhibition, and using the TBA method indicated 17.34% inhibition. Meanwhile, the % inhibition was 23.77% and 40.43% in the nanoparticle sample with the FTC and TBA methods, respectively.
Conclusions: This study revealed that nanoparticles of Sembung leaves can increase antioxidant activities. These findings offer potential applications in the development of existing antioxidant products.

INTRODUCTION

In recent decades, despite significant advancements in science and technology, there has been a lack of improvements in quality of life. Individuals increasingly consume instant foods with inadequate nutritional value, while the widespread habit of smoking, coupled with insufficient physical activities, has contributed to the development of unhealthy lifestyles. Additionally, environments characterised by high levels of pollution have been shown to generate radical compounds that interact with biological systems, causing cellular damage. Air pollution comprises a variety of harmful compounds, including nitrogen oxides, sulphur dioxide, carbon monoxide, and other toxic substances. These compounds are inhaled until they reach the alveoli and enter the bloodstream. Within the human body, these compounds are known to produce Reactive Oxygen Species (ROS), which have been demonstrated to induce cellular damage[1]. The body is able to counteract the effects of these radicals by producing antioxidant compounds that function as antidotes to radical reactions. Sulforaphane (SFN) and its metabolite, sulforaphane N-acetylcysteine (SFNAC), are antioxidants which actively reduce oxidative damage and inflammation[2].

The function of antioxidants in protecting body cells from oxidative damage has been a subject of considerable interest in health and food science research. Free radical-induced oxidative damage has been identified as a fundamental mechanism that contributes to the development of various degenerative diseases, such as cancer, cardiovascular disease, and premature ageing[3–5]. In the era of heightened consumer consciousness regarding health and well-being, there is

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an observed proliferation in demand for food products which are not only nutritious but also have high antioxidant content, thereby conferring augmented health benefits. Natural sources of antioxidants, such as traditional medicinal plants, have been utilised in various medicinal systems worldwide, including in Indonesia, and are now increasingly recognised as potential functional food ingredients[6–8]. The antioxidant and anti-inflammatory properties of *Pterospermum rubiginosum* and *Pterospermum reticulatum*, as evidenced by numerous studies, suggest their potential as functional foods that contribute to health[9–11]. The *Blumea balsamifera* plant, commonly known as the Sembung leaf, has been identified as a natural source of antioxidants. Its utilisation in traditional medicine is attributed to its recognised anti-inflammatory and analgesic properties, in addition to substantiated antioxidant activity[12,13].

Previous studies show that the Sembung leaves contain a various chemical compounds, such as monoterpenes, sesquiterpenes, diterpenes, phenols, flavonoids, triterpenes, cariophyllene oxid, alcohols, and sterols[14,15]. Crude extracts and constituents of this plant have been shown to exhibit a wide range of biological activities, including antioxidant, antimicrobial, antifungal, anti-inflammatory, hypolipidemic, hepatoprotective, antidiabetic, gastroprotective, antitumour, anticancer, and immunomodulatory properties[16]. Flavonoids, categorised within the polyphenol class of antioxidants, function as free radical inhibitors by donating electrons to molecules that have unpaired electrons, without generating new radicals. These mechanisms include decreased production of free radicals such as superoxide and its precursor, hydrogen peroxide, through inhibition of the protein kinase C enzyme, binding of Fe metal ions to inhibit the Fenton reaction, and inhibition of xanthine oxidase enzyme activity. Furthermore, flavanoids have been demonstrated to enhance the activity of endogenous antioxidants, including superoxide dismutase, glutathione peroxidase, and catalase. In the process of lipid peroxidation, flavonoids have been shown to play a role in all stages of initiation, propagation, and termination. They have been demonstrated to block the initiation phase through the inhibition of primary radicals such as superoxide. In the propagation phase, flavonoids react with peroxyl radicals to inhibit the chain reaction. In the termination phase, the radical formed when reacting with peroxyl radicals integrates with other radicals generated during the propagation phase, resulting in a non-radikan product (NRP)[17,18].

A variety of techniques have been developed for evaluating antioxidant activity, including DPPH, CUPRAC and FRAP[19]. Furthermore, the FTC method has been demonstrated to be a reliable approach for determination of antioxidant activity. The FTC method is an early phase of lipid peroxidation, the aim of which is to determine the concentration of peroxides. This is achieved by measuring the formation of ferric-thiocyanate complexes at a wavelength of 500 nm[20]. In this method, unsaturated fats release a hydrogen atom at the methylene group (CH2), resulting in a carbon atom with an unpaired electron (CH). This further triggers the occurrence of propagation reactions during the lipid peroxidation process. Antioxidants function by impeding such chain reactions through the process of hydrogen atom donation[21]. Antioxidants have been shown to reduce free radicals by 50%, as indicated by IC50 values. Therefore, lower IC50 values are linked to higher antioxidant activity[21].

The effective utilisation of Sembung leaf extract as a natural antioxidant is frequently constrained by factors such as the low solubility, stability and bioavailability of its active compounds in conventional formulations, which pose challenges in food applications. The antioxidant activity of Sembung leaves exhibited an IC50 value of 155.65 ml/l. However, the extract of Sembung leaves demonstrated an IC50 value of 293.80 ml/l. These findings suggest that the antioxidant potential of Sembung leaves is within the weak category[22]. Consequently, it is imperative to modify the extract into a nanoparticle preparation of the ethyl acetate fraction of Sembung leaves. The development of nanoparticle technology offers an innovative approach with the potential to enhance the bioavailability and therapeutic effectiveness of active compounds extracted from medicinal plants, including Sembung leaves. The enhanced antioxidant activity observed in the nanoparticle preparation is attributable to the capacity of the nanoparticles to augment the surface area of the ethyl acetate fraction of Sembung leaves. This augmentation facilitates enhanced interaction between the antioxidant compounds and free radicals, thereby augmenting the effectiveness of radical inhibition. Furthermore, it has been demonstrated that nanoparticles can also contribute to an enhancement in the stability of antioxidant compounds. This stability has been demonstrated to be the result of electrostatic interactions between the molecules of chitosan and flavonoid or polyphenol compounds. Chitosan, under conditions of acidity, has positively charged amine groups because of the protonation process (the addition of hydrogen ions). The interaction between these positively charged groups and oxygen atoms in the flavonoid or polyphenol structure tends to be electrophilic. The interaction thus results in a stable bond. As research by Nurviana et al.[23] demonstrated, the highest antioxidant activity was obtained from nanoparticles of *limus* seed ethanol extract. This is indicated by a lower IC50 value when compared to *limus* seed ethanol extract without nanoparticle modification. Statistical analysis showed significant differences in the IC50 values of the samples tested. *Limus* seed extract nanoparticles were more active than *Limus* seed extract and vitamin C. These findings show that nanoparticle preparations can make compounds used as antioxidants more effective in functional food products. Based on these findings, this study aimed to evaluate the antioxidant effectiveness of Sembung leaves in nanoparticle preparations using the FTC method. The application of nanoparticle technology for enriching food products with stable antioxidant compounds has the potential to bring innovation to the food industry, especially in the area of health and disease prevention. Therefore, the study focused on analysing the antioxidant activity of the ethyl acetate fraction of

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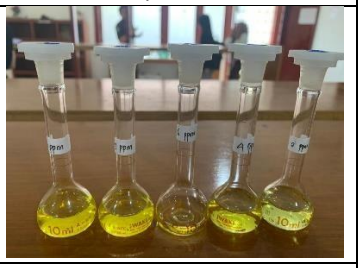
Sembung leaves in nanoparticle dosage form, using the FTC and TBA methods.

METHODS

Materials and Equipments

This research was carried out by utilising various equipment and materials required for laboratory experiments. The equipment employed for this study includes an oven (Mamert), a Spectrophotometer UV-Vis (UV-Mini SHIMADZU), a vacuum rotary evaporator (D lab), an Analytical Balance (A&D Co. LTD), a Vortex (D lab), a Magnetic Stater (SH-2), glassware (Pyrex), and Micropipettes (Socorex). The materials used include Sembung leaves obtained from farmers in Pasaman Regency, West Sumatera, 96% ethanol (Merck), n-hexane (Brataco), ethyl acetate (Brataco), quercetin (Sigma), methanol (Sigma), AlCl3 10% (Sigma), potassium acetate 1M (Sigma), and linoleic acid 2.5% (Sigma), Aquadest, vitamin E (Merck), absolute ethanol (Merck), phosphate buffer, ammonium thiocyanate 30% (Merck), iron (III) chloride solution 0.02 M, analytical reagents, in hydrochloric acid 3.5% (Merck), chitosan (Aldrich), tween-80, Na-TPP (Novalindo), acetic acid 5% (Merck), concentrated HCl (Merck), and thiobarbituric acid (Merck). Figure 1 shows the tools and materials.

		
Sembung leaf	Drying	Dry weight of extract
		
Maceration	Sample rotary process	Condensed extract
		
n-hexane	n-hexane fraction	Ethyl acetate
		
Ethyl acetatefraction	Quercetin mother liquor	Standard quercetin solution

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The sample fraction

Negative control solution

Positive control solution

Figure 1. Stages of sample preparation, equipments and materials

Preparation of Extraction

This study utilised Sembung leaves as the primary sample, which were obtained from the lowland area of Kinali, Bukit Barisan District, West Pasaman, West Sumatera. The plant was identified at the Herbarium of Andalas University. The preparation of the samples involved the division of the Sembung leaves into smaller components, with the objective of achieving the ethyl acetate fraction as the ultimate product. The extraction of leaf pieces was conducted using 96% ethanol to dissolve polar active compounds present in the leaves. The initial partition involved the use of a separating funnel to extract the polar compounds present in the leaves using n-hexane as the extraction solvent. The non-polar compounds were found to dissolve in the upper n-hexane layer, while the polar compounds remained in the lower layer, i.e. the ethanol layer. The n-hexane layer was then separated, and the ethanol layer was subjected to a final partition using ethyl acetate and water. The ethanol layer obtained from the initial partitioning was subsequently subjected to a secondary partitioning process, employing ethyl acetate, water, and ethanol residue, with a volumetric ratio of 100 mL ethyl acetate: 60 mL water: 100 mL ethanol residue. The role of each of these solvents is as follows: ethyl acetate can dissolve semi-polar compounds, water is attracted by highly polar compounds, and the remaining compounds that have not been separated are contained in the ethanol residue. This process was repeated to ensure maximum separation. This stage of the process produced an ethyl acetate fraction containing semi-polar compounds as the final product[24].

Identification of Flavonoid Compounds and Total Flavonoid Content

The presence or absence of flavonoid compounds was determined through the addition of Mg powder and HCl solution to test samples, yielding yellow, brick red and orange colours in a positive test result. The total flavonoid content was determined by preparing a standard solution of quinine, where the quinine standard was dissolved in ethanol to obtain a concentration of 1000 ppm. The quinoline standard solution was initially prepared at a concentration of 1000 ppm, followed by the pipetting of 1 mL of this solution and its dilution in 10 mL of ethanol p.a. to yield a 100 ppm solution. The standard series was then constructed using graded concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm, by pipetting the parent solution in volumes of 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, and 1 mL, respectively. Each quercetin standard solution was then diluted to a volume of 10 mL by the addition of 3 mL of methanol, 0.2 mL of 10% aluminium chloride solution, and 0.2 mL of 1 M potassium acetate solution. Following a 30-minute incubation period at ambient temperature, the wavelength was measured using a spektrophotometer UV-Vis at a wavelength of 445.2 nm[25].

The determination of total flavonoid content was achieved through the dissolution of 100 mg of the ethyl acetate fraction of Sembung leaves into 10 mL of ethanol. The sample solution (1 mL) was then diluted to a total volume of 10 mL by the addition of 3 mL of methanol, 0.2 mL of 10% aluminium chloride solution, and 0.2 mL of 1 M potassium acetate solution. The mixture was then left to incubate at room temperature for 30 minutes to ensure that the reaction was optimal. After this, the sample's optical density was measured using a UV-Vis spectrophotometer. The calculation of compound concentration was based on the light absorbed at a wavelength of 445.2 nm. The analysis was carried out in three replicates to ensure accuracy, with the results of total flavonoid content expressed as quercetin equivalents. The following formula was used to calculate total flavonoid content[25]:

$$\text{Total Flavonoid } \left(\text{mg}\frac{\text{QE}}{\text{g}}\text{extract}\right) = \frac{\text{concentration of quercetin } \left(\frac{\text{mg}}{\text{L}}\right) \text{x volume of extract x dilution factor}}{\text{mass of extract (g)}}$$

Antioxidant Activity Test of Ethyl Acetate Fraction of Sembung Leave

The preparation of the positive control solution (solution A control) involved the use of alpha-tocopherol, also known as vitamin E.A total of 4 mL of absolute ethanol was dissolved in a closed test tube containing 4 mg of vitamin E. Subsequently, 2.5% linoleic acid solution (4.1 mL), 0.05 M phosphate buffer (8 mL), and distilled water (3.9 mL) were added to the mixture. The same procedure was then repeated for the negative control, except for the addition of alpha-tocopherol or vitamin E. Each of solution was prepared in triplicate and subsequently incubated in an incubator at 4°C for a period of 24 hours. Subsequent incubation and measurement of the sample's optical

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density at a wavelength of 500 nm was then conducted.

The preparation and analysis of antioxidant activity was carried out by dissolving 4 mg of the ethyl acetate fraction of Sembung leaf samples in 4 mL of absolute ethanol. The mixture was then added to a 2.5% linoleic acid solution in 4.1 mL increments. To maintain a pH of 7, 8 ml of phosphate buffer (0.05 M ionic strength) and 3.9 ml of distilled water were added. The solution was labelled, and three replicates were made. The mixture was then incubated at 40°C for 24 hours. Afterwards, the absorbances were measured at 500 nm.

Antioxidant activity testing involved solution B (30% ammonium thiocyanate and 9.7 ml of 75% ethanol in a specific volume ratio), added to solution A after a 24-hour incubation. The reaction was continued with the addition of 100 µl of 0.02 M iron (III) chloride solution in 3.5% hydrochloric acid. The mixture was then incubated for five minutes before the absorbances at a wavelength of 500 nm were measured using a UV-Vis spectrophotometer. Absorbance measurements were taken at regular intervals over seven days, or until the negative control solution reached saturation[26]. Thereafter, further analyses were conducted using the TBA (thiobarbituric acid) method.

Antioxidant activity testing involved the TBA method, using FTC test residual samples and standards. Each 2-ml solution was treated with 20% trichloroacetic acid and 0.67% thiobarbituric acid. The mixture was then heated at a set temperature for 10 minutes until boiling. Subsequently, the mixture was subjected to centrifugation at 3000 rpm for 30 minutes. The max wavelength was measured using a UV-Vis spectrophotometer at a wavelength of 500 nm. The percentage inhibition was calculated[27]:

$$\% \text{ inhibition} = \frac{\text{absorbance of blank} - \text{absorbance of sample}}{\text{absorbance of blanko}} \text{ x } 100\ \%$$

Preparation of Nanoparticle

The formulation used in the preparation of nanoparticle is shown in Table 1[28].

Table 1. Formulation of nanoparticle of ethyl acetate fraction of Sembung leaves

Material	F1	F2	F3
Ethyl acetate fraction of Sembung leaves	0.1 g	0.1 g	0.1 g
Chitosan solution 0.25 %	100 mL	-	-
Chitosan solution 0.50%	-	100 mL	-
Chitosan solution 0.75%	-	-	100 mL
NaTPP solution 0.5%	10 mL	10 mL	10 mL
Tween-80	1 mL	1 mL	1 mL

The preparation of ethyl acetate fraction nanoparticles was achieved through the utilisation of an ionic gelation method. Chitosan with varying concentrations, namely 0.25%, 0.50%, and 0.75%, was dissolved in 100 ml of 5% (v/v) acetic acid solution, then homogenised using a magnetic stirrer. A Na-TPP solution with a concentration of 0.5% was prepared in aquadestilate. The next step was to combine the chitosan solution with 1 ml of tween-80 in an Erlenmeyer flask and homogenise it to form a stable emulsion for 10 min using a magnetic stirrer at 1000 rpm. In the subsequent step, 0.1 g of the ethyl acetate fraction of Sembung leaves was added to the solution and homogenised again for 30 minutes at 1,400 rpm. Subsequently, 0.5% Na-TPP solution was added and the mixture was homogenised for 120 minutes at 1400 rpm. The final product was then left to stand for 24 hours[28]. Following this, the samples were characterised using a Particle Size Analyzer (PSA) to determine the particle size. The nanoparticle formulations that had been formed were then analysed for antioxidant activity using the same method as in the previous test.

RESULTS AND DISCUSSIONS

The leaves of the Sembung plant used in this study were obtained from the Kinali area, Pasaman Regency, West Sumatra, Indonesia. These leaves then underwent a process of determination to ensure the accuracy of identification and to prevent errors in the analysis of samples. The determination process, which was carried out at the Herbarium laboratory of Andalas University, Padang, confirmed that the sample was a Sembung plant with the Latin name *Blumea balsamifera (L) DC*. During the preparation stage, the leaves were dried without direct exposure to direct sunlight. This approach was adopted to mitigate potential damage to active compounds due to ultraviolet radiation. This approach is informed by extant studies demonstrating that exposure to sunlight during the drying process can lead to a significant decline in the levels of phenolic acids (up to 29%) and flavonoids (up to 86%)[29]. So a more meticulous and controlled drying method was done to ensure the preservation of bioactive compounds.

The extraction process used the maceration method in which the sample was soaked in organic solvent. This method facilitates the disruption of plant cell walls and membranes, thereby enabling the dissolution of secondary metabolite compounds contained within the cytoplasm into the solvent. The solvent employed was 96% ethanol, based on the principle of like dissolves like, given the organic nature of secondary metabolite compounds and their enhanced solubility in organic solvents. Ethanol solvents are favoured over other organic solvents due to their neutrality, high absorption capacity, non-toxicity, and resistance to microbial and fungal growth. These

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properties ensure that the extraction process yields optimal and safe results[30].

The maceration of 500 g of the sample yielded 7.2 g of extract, which exhibited a green colour and a distinctive aroma of Sembung leaves, with a yield of 14.4%. At the multistage fractionation stage, n-hexane and ethyl acetate solvents were utilised to separate the extract into three fractions: n-hexane condensed fraction, ethyl acetate fraction, and ethanol residual fraction. The flavonoid identification test revealed that the ethyl acetate fraction was positive for flavonoids, as evidenced by the colour change of the sample from green to yellow following the addition of concentrated HCl, indicating the presence of flavones, chalcones, and aurones. The phytochemical profile indicated that the ethyl acetate fraction contained significant concentrations of phenolic compounds, alkaloids, and flavonoids. Consequently, these results indicate that the ethyl acetate fraction is a potential source of bioactive compounds. Conversely, the n-hexane and residual water fractions exhibited a more restricted compound profile, characterised by the presence of steroids and saponins, respectively. The presence of phenolic compounds, alkaloids, and flavonoids in the ethyl acetate fraction indicated higher antioxidant potential. In view of the more complex and diverse secondary metabolite content in the ethyl acetate fraction, this fraction was selected as the primary focus in the evaluation of antioxidant activity and quantification of specific flavonoid content.

Flavonoids, a group of phenolic compounds, possess significant antioxidant activity which contributes to the protection of the body from free radical damage. Free radicals have been demonstrated to induce various degenerative diseases through oxidative mechanisms. The quantification of total flavonoid content in the extract was accomplished by selecting UV-Vis spectrophotometric analysis as the method of choice. The presence of conjugated aromatic functional groups on flavonoids increases their strong absorption in the ultraviolet-visible region of the electromagnetic spectrum. The interaction between flavonoids and aluminium chloride ions forms a yellow complex that results in a shift of the maximum wavelength of absorption to the visible spectrum. Potassium acetate was also incorporated to maintain the wavelength in the visible spectrum. The incubation stage is integral to ensure that the complex formation reaction between flavonoids and aluminium chloride ions is complete, thereby ensuring the intensity of the colour formed reaches its maximum level prior to measurement[31].

The quantification of total flavonoids by colorimetry is based on the formation of complexes with aluminium ions, which results in a wavelength shift to the visible spectrum. This is marked by a change in the colour of the solution to a more yellow hue. This reaction occurs between AlCl3 and the ketone group at C4 and the hydroxyl group (OH) at position C5 of the flavonol compound, resulting in a stable complex compound[32]. The addition of potassium acetate has been demonstrated to play a role in stabilising the complex compound. The study revealed that the total flavonoid content in the ethyl acetate fraction of Sembung leaves (Blumea balsamigfera L.) averaged 31.903 mg QE/g extract. This value indicates the amount of flavonoids contained in each gram of sample. Detailed data on total flavonoid levels can be found in Table 2.

Table 2. Todal flavonoid content of ethyl acetate fraction of sembung leaf

Repitation	Absorbance	Concentration (mg/L)	Total Flavonoid (mg QE/ g Extract)
P1	0.697	6.269	31.360
P2	0.695	6.253	31.270
P3	0.736	6.616	33.080
			31.903

The flavonoid content of plants is subject to considerable variation, influenced by a variety of factors including genotype, geographical location, developmental stage, harvesting methodology, and post-harvest storage conditions. These factors have the capacity to affect both the total flavonoid concentration and the composition of flavonoids present in the plant[33]. The antioxidant activity of the ethyl acetate fraction was analysed using the FTC (Ferri Thiosianat) and TBA (Thiobarbituric Acid) methods, as shown in Table 3 and 4.

Table 3. The average absorbance value of ethyl acetate fraction samples using FTC method

Days	Control (-)	Control (+)	Ethyl Acetate Fraction	% Inhibition
1	2.214	1.485	1.141	48.46
2	2.602	1.822	1.559	40.08
3	2.812	2.069	1.740	38.12
4	2.863	2.181	1.814	36.63
5	2.921	2.232	1.997	31.63
6	3.209	2.553	2.206	31.25
7	3.134	2.448	2.216	29.29

Note:
Control (+)/positive = Vitamin E
Control (-)/negative = without vitamin E

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Table 3 shows that the negative control exhibited a decrease in the absorbance value on day 7, as measured by a UV-Vis spectrophotometer at a wavelength of 500 nm. This finding indicates that the lipid peroxidation reaction attained a saturation point on day 6, with an inhibition efficiency of 31.25%. This percentage inhibition value indicates the ability of the test compound to inhibit the formation of hyperoxide radicals at the propagation stage of the lipid peroxidation reaction.

Table 4. Inhibition percentage of ethyl acetate fraction of sample using TBA method

Control (-)	% Inhibition of Sembung Leaf Ethyl Acetate Fraction		
Mean Abs	Replica 1	Replica 2	Replica 3
1.07	18.97	16.35	16.72
	Average	17.34	

The FTC (Ferri thiocyanate) and TBA (thiobarbituric acid) methods utilised in this study are spectrophotometer methods that are frequently employed to evaluate antioxidant activity in indirectly inhibiting free radicals. This test is performed using a single concentration of extract or sample, and the free radicals inhibited include peroxide compounds and malonaldehyde compounds. The formation of these compounds necessitates meticulous experimental control to ensure the accuracy of the results. The results obtained from the study indicated that the ethyl acetate fraction exhibited 31.25% inhibition in the FTC method on the sixth day, while the TBA method recorded an average inhibition of 17.34%. This inhibition value is indicative of the ability of antioxidants to neutralise free radicals at a given concentration of test solution.

In order to enhance the antioxidant activity of the ethyl acetate fraction, it is formulated into nanoparticle preparations. Nanoparticles are nanometer-sized preparations, specifically betwen 1 and 100 nm. This is done as the very small particle size can increase the absorption of antioxidant compounds contained in the sample. The reduction in particle size leads to an augmentation in surface area, thereby enhancing solubility. This, in turn, results in an increase in bioavailability and efficacy. The formulation employed in the synthesis of the ethyl acetate fraction into nanoparticle preparations is outlined in Table 1. The method of nanoparticle fabrication is ionic gelation, which involves cross-interaction between polyelectrolytes and multivalent ion pairs. Chitosan was selected as the nanocarrier or carrier polymer due to its biocompatibility, biodegradability, and non-toxicity. The characterisation of the nanoparticle was performed using a PSA (Particle Size Analyser), a device designed to measure particle size distribution on a nanometer scale. The working principle is based on the scattering of laser light by particles in the sample. The light is emitted through a single point hole directed at the particles in the sample, which subsequently scatter the light back through the point hole towards the detector. The analogue signal that is thus detected is converted into a digital signal, and further converted into a counting series[34].

The mean particle size obtained for F1 was 126.7 nm, for F2 was 40.81 nm, and for F3 was 259,6 nm. It is therefore concluded that the nanoparticle formula that meets the standard requirements of nanoparticle size below 100 nm is formula F2. It is acknowledged that the variation in the concentration of chitosan and Na-TPP, which functions as a carrier agent for nanoparticle reactions, affects the results of the acquisition of nanoparticle size. Furthermore, the F2 nanoparticle preparation was continued for antioxidant activity analysis using the same method when analysing the previous extract. The results obtained can be seen in Tables 5 and 6.

Table 5. Average Absorbance Value of F2 nanoparticles using FTC method

Days	Control (-)	Control (+)	Nanoparticle F2	% Inhibition
1	2.214	1.485	0.787	64.45
2	2.602	1.822	1.738	52.22
3	2.812	2.069	1.945	53.20
4	2.863	2.181	2.063	45.96
5	2.921	2.232	2.151	40.48
6	3.209	2.553	2.466	23.77
7	3.134	2.448	2.369	34.93

Note: Control (+) = Vitamin E
Control (-) = without vitamin E

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Table 6. Inhibition percentage of F2 nanoparticles using TBA method

Control (-)	% Inhibition of F2 Nanoparticles		
Mean Abs	**Replica 1**	**Replica 2**	**Replica 3**
1.07	40.56	37.75	42.99
	Average	40.53	

The findings of the research indicate that on average, the ethyl acetate fraction nanoparticle sample exhibited 39.63% inhibition on the sixth day, while the TBA method recorded an average inhibition percentage of 40.43% for the ethyl acetate fraction nanoparticle preparation. The findings of this study indicate that the formulation of samples as nanoparticles can enhance their antioxidant activity.

Antioxidant activity testing using the FTC method is based on the ability of antioxidant compounds to inhibit colour formation, which is reflected by their capacity to maintain absorbance values. The FTC method quantifies the amount of peroxides formed in the initial stages of lipid oxidation, designated as primary products. Peroxide formation progresses until it reaches a maximum limit, after which a decrease (decomposition) occurs. In the FTC method, the peroxide formed from linoleic acid reacts with Fe^{2+}, producing Fe^{3+}. This integrates with thiocyanate to form a red-coloured complex. Concurrently, the TBA method is utilised to quantify the amount of MDA formed, in addition to evaluating the extent of lipid peroxidation. Under conditions of low pH and elevated temperature, MDA reacts with TBA to form a pink-coloured complex. MDA is the primary product of carbonyls in the process of autooxidation of unsaturated lipids. The objective of this evaluation is to ascertain the quantity of MDA compounds that have been formed. The formation of this compound occurs during the second stage of lipid peroxidation. The percentage of inhibition obtained indicates the ability of the antioxidant compounds present in the sample to capture free radicals at the concentration of the test solution.

This study proposes a novel approach to enhance the antioxidant activity of Sembung (*Blumea balsamifera* L.) leaves through the utilisation of an ethyl acetate fraction-based nanoparticle formulation. The significance of this study lies in the use of FTC and TBA methods for the comprehensive measurement of antioxidant effectiveness, enabling evaluation at both the early and advanced stages of lipid peroxidation. The nanoparticle modification yielded significant results, with enhanced antioxidant activity supported by higher percentage inhibition measurements compared to conventional extracts. The use of chitosan as a carrier polymer in the ionic gelation method also provided advantages in terms of stability and bioavailability of the active compounds. Despite the study's notable contributions, it is important to acknowledge its limitations. For instance, the testing was conducted at a single concentration of extract and nanoparticles, precluding a comprehensive exploration of dose variations. Consequently, the study was unable to ascertain the most efficacious dose range to achieve maximum antioxidant activity.

CONCLUSIONS

The modification of Sembung leaf extract (*Blumea balsamifera* L.) into nanoparticle form has been shown to yield a significant increase in antioxidant activity when compared to conventional extracts. Tests using ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods demonstrated that the ethyl acetate fraction nanoparticle preparation of Sembung leaves exhibited superior potential in preventing cell damage caused by free radicals. The percentage inhinhibition for FTC and TBA was 23.77% and 40.43%, respectively, compared to the conventional ethyl acetate fraction extract, which showed percentage inhibition of 31.25% and 17.34%, respectively. This increased activity is likely due to the smaller particle size of the nanoparticles, which increases the contact surface area between the antioxidant compounds and the free radicals, as well as improves the stability and bioavailability of the active compounds.

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CONFLICT OF INTEREST AND FUNDING DISCLOSURE

All authors have no conflict of interest in this article.

AUTHOR CONTRIBUTIONS

IAS: final writing, revision, review, and editing original draft; AR: conceptualization, reseach methodology, supervision, writing original draft; TA: investigation, supervision, review; WUZ: formal analysis.

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