

Phytochemical Characterization and Nano-/Submicron Emulsion Formulation of Merbau Wood Extract with Nutmeg Leaf Essential Oil: Physicochemical Properties and *in Vitro* Bioactivity

Ratih Afrida Lismana Sari¹, Manggar Arum Aristri², Maeda Wahyuningrum³, Mellania Chandra Dewi⁴, Niken Hardiyanti⁵, Dhimas Pramadhani⁶, Awanda Wira Anggini⁶, Rita Kartika Sari^{6*}, Yanico Hadi Prayogo⁶ and Sunardi^{7,8}

¹Faculty of Forestry, Lambung Mangkurat University, Banjarbaru, South Kalimantan, Indonesia

²Department of Forest Management, Faculty of Agriculture, Sebelas Maret University, Surakarta, Indonesia

³Department of Forestry Engineering, Institut Teknologi Sumatera (ITERA), South Lampung, Indonesia

⁴Department of Biochemistry, Faculty of Mathematics and Natural Sciences, IPB University, Bogor, Indonesia

⁵School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia

⁶Department of Forest Product, Faculty of Forestry and Environment, IPB University, Bogor, Indonesia

⁷Wetland-Based Materials Research Center, Lambung Mangkurat University, Banjarbaru, South Kalimantan, Indonesia

⁸Department of Chemistry, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, Banjarbaru, South Kalimantan, Indonesia

*Corresponding author (e-mail: rita_kartikasari@apps.ipb.ac.id)

The potential of merbau extract as a natural skin lightener and nutmeg leaf essential oil as an aromatic agent in cosmetics is well-recognized, yet their direct application faces bioavailability challenges due to the lipid-rich, non-polar nature of the stratum corneum; overcoming this skin barrier therefore requires an advanced formulation capable of enhancing ingredient solubility and improving dermal delivery. This study was designed to quantify the yield and characterize the phytochemical constituents of merbau extract and nutmeg leaf oil, and to identify the physicochemical, antioxidant, and antityrosinase properties of a nanoemulsion incorporating this combined active blend. Merbau extract and nutmeg leaf essential oil were obtained through Ultrasound-Assisted Extraction (UAE) and steam distillation, respectively, after which a spontaneous emulsification technique was used to formulate the nanoemulsion; both the pure extracts and the resulting formulations were then subjected to laboratory analysis. Analytical profiling showed that merbau extract is rich in robidanol, artemidiol, sakuranetin, sayanedin, and 4-methyl-6,7-dimethoxycoumarin (via LC-MS), while nutmeg oil is dominated by myristicine, cyclotrisiloxane, 2-pinene, 1,4-cyclohexadiene, and 1,3-benzodioxole (via GC-MS). The raw merbau extract exhibited antioxidant and antityrosinase activity at 11.25 ppm and 6.82 ppm, respectively, and among the tested formulations, Formula 3 containing a 30% active ingredient concentration performed best, yielding a droplet size of 590.4 nm along with antioxidant and antityrosinase activities of 19.40 ppm and 11.43 ppm. These findings indicate that fabricating a nanoemulsion from merbau extract via UAE effectively overcomes initial bioavailability limitations, with Formula 3 showing the optimum combination of particle size and bioactivity, representing a promising candidate base for natural skin-lightening formulations, pending further dermatological and toxicological safety evaluation.

Keywords: Antityrosinase, merbau, nanoemulsion, nutmeg leaves, uae

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Skin exposure to high levels of ultraviolet (UV) radiation is a common environmental challenge in many regions of the world, particularly in areas with tropical and subtropical climates. Continuous UV exposure triggers excessive melanin production through the upregulation of tyrosinase enzyme activity, leading to hyperpigmentation and uneven skin tone [1]. These conditions often affect self-confidence and drive the widespread use of skin-lightening cosmetic products [2]. However, many commercially available skin-lightening agents such

as hydroquinone and kojic acid, which carry the risk of potential side effects of potential side effects, including cytotoxicity and environmental harm [3, 4]. This has increased global interest in safer, plant-derived bioactive compounds, although the scientific evidence supporting the efficacy of many natural-based cosmetic preparations remains limited.

Merbau wood (*Intsia* spp.) is known to contain abundant extractive compounds such as robidanol and its derivatives, which exhibit strong tyrosinase-inhibitory activity, even surpassing that of kojic acid

[5, 6]. For instance, 50% ethanol extracts of *Intsia* sp. have shown potent antityrosinase activity *in vitro* (reported IC₅₀ values in low ppm range), supporting their potential as skin-lightening actives [7]. Despite this potential, merbau extracts—typically obtained using polar solvents show limited dermal penetration because the stratum corneum is predominantly lipophilic, resulting in low bioavailability [8]. In parallel, the wood-processing industry generates substantial merbau residues (e.g., sawdust and milling waste) that remain underutilized [9], even though these materials are rich sources of phenolic and flavonoid compounds. Valorizing these residues as high-value cosmetic bioactives offers an opportunity to support sustainable forestry and bioproduct development.

In addition to the active component, natural fragrances are increasingly preferred over synthetic ones in cosmetic formulations [10, 11]. Nutmeg leaf essential oil, which contains characteristic aromatic compounds such as sabinene and myristicine, represents a renewable yet underexplored natural fragrance source because most research focuses on nutmeg seeds or mace [12, 13]. Integrating nutmeg leaf essential oil into cosmetic preparations may enhance product acceptability while maintaining natural and biodegradable profiles. Beyond its olfactory function, nutmeg leaf essential oil was also incorporated based on literature reports describing antioxidant and antityrosinase-related bioactivities associated with its major constituents, including sabinene, terpinene-4-ol, myristicine, and elemicin [14]. These compounds have been suggested to contribute to radical-scavenging activity and inhibition of melanogenesis-related enzymes in previous studies, providing the scientific rationale for selecting this essential oil as a functional component of the formulation rather than relying solely on its fragrance value.

Given the polarity mismatch and low bioavailability of merbau extract, an advanced delivery system is needed to ensure optimal skin penetration. Nanoemulsions are widely applied in dermatology due to their small droplet size, improved stability, enhanced solubility of active compounds, and superior dermal delivery properties [15]. However, studies on nanoemulsions combining merbau wood extract with nutmeg leaf essential oil as a natural skin-lightening composition have not yet been reported. Therefore, this study aims to characterize the phytochemical profiles of merbau wood extract and nutmeg leaf essential oil, and to develop and evaluate nanoemulsions for their physicochemical properties as well as antioxidant and antityrosinase activities.

EXPERIMENTAL

Material

The materials used in this study included merbau wood powder and nutmeg (*Myristica fragrans*) leaves as the primary raw materials. Ethanol (96%) and methanol (Merck, Germany) were utilized as extraction solvents, while distilled water was used for solution preparation. Anhydrous Na₂SO₄ was applied as a drying agent. Tween 80, glyceryl caprylate, and propylene glycol were used as formulation components. DPPH (2,2-diphenyl-1-picrylhydrazyl) (Merck, Germany) was employed for antioxidant activity analysis. In addition, a tyrosinase enzyme solution, phosphate buffer, and L-tyrosine substrate solution were used for the tyrosinase inhibition assay, with kojic acid serving as a positive control. The equipment used included a Willey mill for grinding the raw materials, followed by a 40–60 mesh sieve to obtain uniform particle size, and a mortar for additional manual homogenization. An analytical balance and digital scales were used to measure sample and reagent masses, while calipers were used for dimensional measurements. A microwave oven was utilized to assist the extraction process. Erlenmeyer flasks and measuring flasks were used for solution preparation, and a magnetic stirrer was applied to ensure homogeneous solutions. A particle size analyzer (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK) was used to determine particle size distribution, and a pH meter was used to measure solution acidity. Test tubes served as reaction vessels, while an incubator was used to maintain controlled temperature conditions. A 96-well microplate and microplate reader were used for assay measurements, and a UV-Vis spectrophotometer was employed to determine absorbance values.

Method

This study was carried out through a series of systematically designed stages, as outlined in the research workflow presented in Figure 1. Initially, simplicia were prepared, followed by solvent extraction and comprehensive physicochemical characterization of the resulting extract. The extract was subsequently incorporated into a nanoemulsion using appropriate formulation techniques. The nanoemulsion was then subjected to detailed physicochemical and stability evaluations to identify the optimal formulation parameters. The selected nanoemulsion was further utilized as an active ingredient in the development of a topical cream formulation. Finally, the prepared cream underwent a series of evaluation tests, including physicochemical and performance assessments, to determine its suitability and functional efficacy.

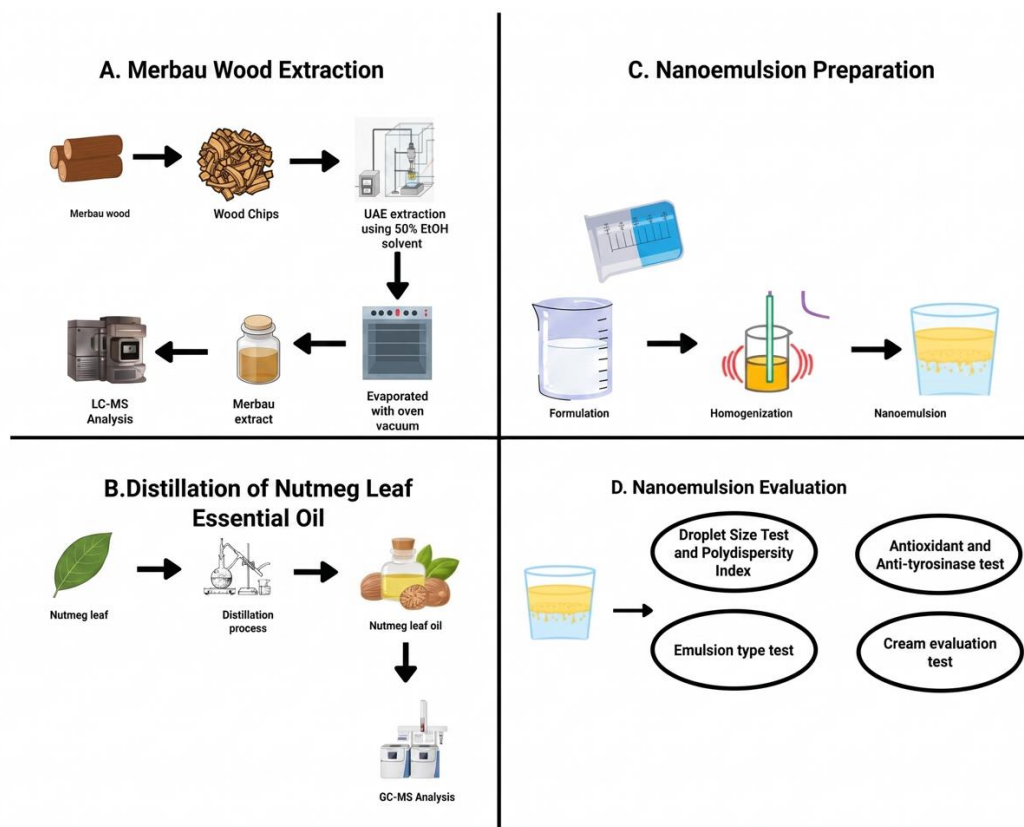


Figure 1. Schematic representation of the research workflow.

Merbau Wood Extraction

A total of 5 g of merbau powder was subjected to extraction using the ultrasound-assisted extraction (UAE) method with 50% (v/v) ethanol as the solvent at a solid-to-solvent ratio of 1:10 (w/v). The mixture was exposed to ultrasonic irradiation at an amplitude of 65% for 45 min. The residual biomass was subsequently re-extracted using 50 mL of fresh solvent under identical conditions. This extraction procedure was repeated three times to ensure maximum recovery of extractable compounds. These combined extracts were filtered to remove insoluble residues, and the filtrates were pooled and concentrated using a vacuum oven until a constant weight was achieved. The extraction yield was then determined gravimetrically. Phytochemical profiling of the obtained extract was performed using liquid chromatography-mass spectrometry (LC-MS).

Essential Oil Extraction from Nutmeg Leaves

A total of 500 g of fresh nutmeg leaves were subjected to essential oil extraction via water and steam distillation. The distillation process was conducted at 100-105 °C for 6 h. The obtained distillate was subsequently treated with anhydrous Na₂SO₄ to remove residual moisture and yield the essential oil

fraction. The chemical composition of essential oil was then characterized using gas chromatography-mass spectrometry (GC-MS).

Nanoemulsion Preparation

The nanoemulsion was prepared using a high-energy emulsification approach. Merbau extract, essential oil, glyceryl caprylate, propylene glycol, and glycerin were initially combined in a baker and homogenized using a magnetic stirrer at 500 rpm and 50 °C for 15 min. Subsequently, Tween 80 was added to the mixture, and the stirring speed was increased to 700 rpm while maintaining the temperature at 50 °C for an additional 10 min until a homogeneous oil phase was obtained (mixture 1). Distilled water was then gradually introduced into mixture 1 under continuous stirring at 700 rpm and 50 °C for 10 min to form a coarse emulsion. After achieving homogeneity, the heating was discontinued while stirring was maintained at 700 rpm for a further 10 min to stabilize the system. The emulsion was subsequently subjected to ultrasonic treatment for 3 min to reduce droplet size and enhance nanoemulsion formation. The resulting nanoemulsion was prepared in three different formulations to determine the optimal composition, as presented in Table 1.

Table 1. Nanoemulsion of merbau extract and essential oil.

Materials	Concentration of each ingredient (%)		
	F1	F2	F3
Merbau extract	10	20	30
Essential oil	5	5	5
Glyceryl caprylate	10	10	10
Propylene glycol	25	25	25
Glycerin	20	20	20
Tween 80	3	3	3
Distilled water	Ad 100	Ad 100	Ad 100

Nanoemulsion Evaluation

The nanoemulsion was evaluated through a series of physicochemical and biological analyses to assess its stability and functional performance. Droplet size and polydispersity index were determined using a particle size analyzer (PSA) to evaluate particle distribution uniformity and system stability. The emulsion type was identified using the methylene blue test, where uniform dispersion of the dye indicated an oil-in-water (O/W) system, whereas the presence of discrete blue droplets suggested a water-in-oil (W/O) emulsion. Antioxidant activity was assessed using the DPPH radical scavenging assay, based on the reduction of DPPH radicals as indicated by a color change from purple to yellow. The antioxidant capacity was expressed as the percentage inhibition of DPPH absorbance. The antityrosinase assay was performed following the method of Sari et al. [5] with slight modifications. The assay was conducted in a 96-well microplate by sequentially adding 80 µL of phosphate buffer solution (50 mM, pH 6.5), 40 µL of L-tyrosine substrate solution, 40 µL of tyrosinase enzyme solution, and 40 µL of the test sample (merbau extract, essential oil, or nanoemulsion). This assay employed the monophenolase reaction using L-tyrosine as the substrate. The concentration range of the test samples was 0.98 to 62.50 ppm. After thorough mixing, the reaction mixture was incubated at 25–30 °C for 30 minutes in the dark, after which the absorbance of each reaction mixture was measured at 510 nm using a microplate reader. This wavelength corresponds to the maximum absorbance of dopachrome, the colored product of L-tyrosine oxidation by tyrosinase. Four solutions were included in each assay: the sample solution (sample + enzyme + substrate + buffer), the sample control solution (sample + buffer, without enzyme and substrate; serving as a correction factor), the blank solution (enzyme + substrate + buffer, without sample or inhibitor), and the blank control solution (buffer only, without sample, enzyme, or

substrate). Kojic acid, which served as the positive control, underwent the same assay procedure across the same concentration range. The percentage of tyrosinase inhibitory activity was calculated using the following equation: $\text{probit\% Antityrosinase activity} = [(A-B)-(C-D)] / (A-B) \times 100\%$, where A is the absorbance of the blank solution, B is the absorbance of the blank control solution, C is the absorbance of the sample solution, and D is the absorbance of the sample control solution. The IC₅₀ value was subsequently determined from the dose–response relationship by non-linear regression analysis. All measurements were performed in triplicate (n = 3).

Data Analysis

Data were analyzed using a completely randomized design with three nanoemulsions as treatment groups. Three independently prepared formulation batches were analyzed (n = 3; experimental replicates). Statistical analysis was performed using one-way analysis of variance (ANOVA) to determine significant differences among treatments. When significant effects were observed ($p < 0.001$), Duncan's multiple range test (DMRT) was applied as a post hoc analysis. All statistical analyses were conducted using SPSS software. The analysis covered particle size, polydispersity index, antioxidant activity, and antityrosinase activity.

RESULTS AND DISCUSSION

Extract Yield and Its Characterization

The moisture content of the prepared merbau simplicia was 9.86%, which met the quality standard of <10%. Water content that is too high (> 10%) causes the growth of microbes that will reduce the stability of the extract [16]. The yield and characteristics of the merbau extract are presented in Table 2.

Table 2. Yield and physicochemical characteristics of merbau extract.

Indicators	Result
Colour	Reddish brown
Form	Solid
Aroma	Unscented
Yield (%)	13.69
Visual appearance	

The extraction yield of merbau wood obtained using 50% ethanol (E50%) via the UAE method was notably higher (13.69%) than that of the methanolic extract produced through maceration, which yielded 4.66% [16]. This variation can be attributed primarily to differences in solvent polarity. Furthermore, the extraction technique employed also plays a significant role in influencing the overall yield. Ultrasound-Assisted Extraction (UAE) has been demonstrated to produce significantly higher extract yields and richer phytochemical contents compared to conventional maceration, along with shorter extraction times and better preservation of antioxidant activity [17–19]

Essential oils (EOs) are volatile compounds naturally derived from various plant parts, such as flowers, buds, stems, twigs, fruits, seeds, roots, bark, and leaves [20–22]. A range of extraction techniques has been developed, among which

hydrodistillation and steam distillation are considered the most traditional and widely applied methods. In addition, dry distillation is often employed, particularly for obtaining oils from woody materials. Within the cosmetic and skincare industries, essential oils are primarily valued for their characteristic and appealing aromas. In this study, essential oil from fresh, chopped nutmeg leaves was obtained using the steam distillation method. The yield and characteristics of the resulting oil are summarized in Table 2. The yield obtained (0.66%) was higher than that reported in a previous study (0.52%) [23]. Variations in essential oil yield can be influenced by multiple factors, including geographical origin and growing conditions. The nutmeg leaves used in the present study were sourced from Bogor, whereas the comparative study [23] utilized samples from Lemukutan Island, which may account for the observed differences.

Table 3. Yield and physicochemical characteristics of nutmeg leaf essential oil.

Indicators	Result
Colour	Clear yellow
Form	Liquid
Aroma	Spicy nutmeg aroma
Yield (%)	0.66
Density (g/mL)	0.92
Mass of oil (g)	15.82
Visual appearance	

Phytochemical Profile, Antioxidant Capacity, and Antityrosinase Activities of Merbau Extract

LC–MS analysis (Figure 2) identified a total of 19 compounds in the merbau wood extract, with robidanol being the predominant constituent. This compound has been reported to possess inhibitory activity against the tyrosinase enzyme [5]. The antioxidant evaluation demonstrated that the extract exhibited a strong radical-scavenging capacity, with an IC_{50} value of 11.25 ppm, thereby classifying it as a highly potent antioxidant [24]. Furthermore, the antityrosinase assay revealed that the extract showed significant inhibitory activity, with an IC_{50} value of 6.82 ppm. This activity was notably stronger than that of kojic acid, which exhibited an IC_{50} value of 33.24 ppm.

Merbau extract is dominated by phytocompounds belonging to the flavonoid group. Based on LC-MS analysis, several classes of compounds were identified, namely flavonoids, coumarins, amines, amino acids, lipids, and esters. In total, the percentage of each identified compound class was 57.14% (flavonoids), 20.29% (coumarins), 12.23% (amines and amino acids), 5.34% (lipids), and 3.95% (esters). Robidanol showed a higher relative abundance compared to other flavonoid compounds (Table 4). Four other compounds with high relative abundance were artemidiol, sakuranetin, sayanidine, and 4-methyl-6,7-dimethoxycoumarin.

Phytochemical and Antioxidant Activity of Nutmeg Leaf Essential Oil

The chemical components of nutmeg leaf essential oil are highly diverse. In total, 147 compounds were

identified, and 21 of them showed higher relative abundance compared to the others (Table 5). There were five dominant compounds, defined as those with peak % abundance values higher than the remaining compounds. These five highest peaks appeared at retention times of 3.47, 12.18, 7.84, 6.81, and 8.83 minutes, respectively. The compound detected at 3.47 minutes, with the highest relative abundance of 4.82%, was 2-pinene. This finding is supported by [13], who also reported pinene as the compound with the highest relative abundance (26). Other compounds with high relative abundance included eicosane (2.86%), phenol (2.55%), 1,3-benzodioxole (2.26%), and myristicine (2.24%). These results differ from previous studies indicating that nutmeg leaf essential oil is dominated by myristicine [25]. It is important to note the detection of cyclotrisiloxane (1.41%). In phytochemistry, siloxane derivatives are universally recognized as analytical artifacts rather than genuine plant metabolites. The presence of this compound is attributed to background contamination, most likely originating from GC-MS column bleed (stationary phase degradation) or silicone septum leaching during high-temperature injection. As an analytical artifact, it does not contribute to the biological activity of the essential oil and was excluded from the assessment of the formulation's efficacy.

The essential oil must be stored in a tightly sealed and dark container to protect its main active compound responsible for antioxidant activity from light exposure. Light can induce compositional changes, oxidation reactions, and degradation of certain compounds, ultimately reducing antioxidant content [25–27]. The antioxidant activity test of nutmeg leaf essential oil showed no detectable activity even at concentrations up to 1000 ppm.

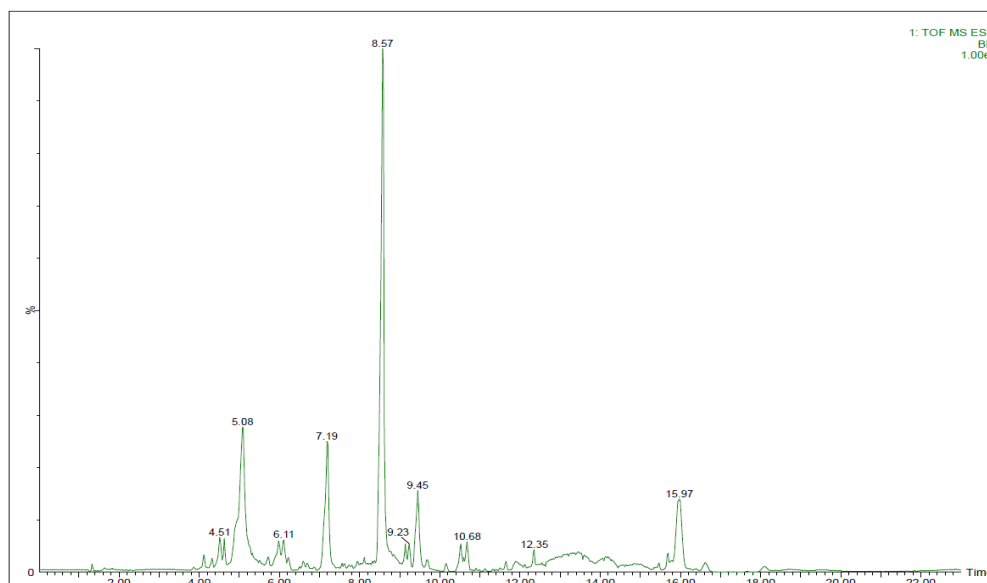


Figure 2. Chromatogram analysis of merbau extract.

Table 4. Chemical constituents of merbau extract.

RT	m/z	Identified Compound	Relative abundance (%)	Group of Compound
5.077	317.0654	Robidanol	17.35	Flavonoid
8.593	235.0969	Artemidiol	9.78	Coumarin
9.141	287.0919	(±)-Sakuranetin;	9.78	Flavonoid
9.254	299.0918	Syanedine;	9.78	Flavonoid
7.186	221.0818	4-Methyl-6,7-dimethoxycoumarin	7.79	Coumarin
15.954	394.3468	Dioctyldiphenylamine	7.79	Amine
9.471	301.1075	4'-Hydroxy-5,7-dimethoxyflavanone	5.43	Flavonoid
5.697	287.0557	Fisetin	4.73	Flavonoid
5.977	319.0453	Myricetin	4.73	Flavonoid
13.317	496.3401	Arginyl-prolyl-lysyl-prolinamide	4.44	Amino acid
13.317	615.4984	Diacylglycerol	4.44	Lipid
4.508	449.1078	Quercitrins	3.21	Flavonoid
10.505	496.1972	Dimethyl 1-[2-(4-methoxyphenyl)ethyl]-4-(3,4,5-trimethoxyphenyl)-1,4-dihydro-3,5-pyridinedicarboxylate	2.72	Ester
10.660	221.0814	4-Methyl-6,7-dimethoxycoumarin;	2.72	Coumarin
12.334	553.4252	Benzyl (2S,3S,5S)-3-(benzyloxy)-2-hexyl-5-hydroxyhexadecanoate	1.23	Ester
4.115	595.1445	Piperitoside	0.84	Flavonoid
16.657	429.3722	(-)-Cholesteryl acetate	0.69	Lipid
6.596	289.0712	Eriodictyol	0.57	Flavonoid
8.155	317.1021	Melilotocarpan D	0.36	Flavonoid
7.940	273.0763	Naringenin	0.36	Flavonoid
10.153	343.2955	Lauramidopropylbetaine	0.21	Lipid

Characteristics of Nanoemulsion

Visual evaluation of the formulated oil-in-water (O/W) nanoemulsions, which incorporate active ingredient concentrations of 10% (F1), 20% (F2), and 30% (F3), revealed a distinct black coloration (Figure 3).

The results of the emulsion type test for all formulations are shown in Figure 4 (F1, F2, and F3). Visual and microscopic observations confirmed that all three formulations formed oil-in-water

(O/W) nanoemulsions. This O/W characteristic is marked by the blue dye evenly dispersing throughout the continuous phase [28]. Nanoemulsions are defined as transparent or translucent O/W systems with droplet sizes ranging from 20 to 200 nm [29]. They constitute heterogeneous, thermodynamically stable mixtures of immiscible liquids (oil and water), whose stability is maintained by an interfacial layer of surfactants and cosurfactants. The primary factor preventing droplet coalescence is the Brownian motion of the dispersed phase [30].

Table 5. Chemical constituents of nutmeg leaf essential oil.

No	Retention time (mins)	Identified compound	Relative abundance (%)
1	3.47	2-Pinene	4.82
2	12.18	Eicosane	2.86
3	7.84	Phenol	2.55
4	6.81	1,3-Benzodioxole	2.26
5	8.83	Myristicine	2.24
6	4.52	Cyclohexene	1.93
7	11.65	Hexadecanoic acid	1.89
8	15.26	1H-indene	1.8
9	6.33	2,6-Octadienal	1.74
10	20.08	(-)-18-noramborx	1.74
11	4.68	1,4-Cyclohexadiene	1.58
12	8.61	1H-Cycloprop[e]azulene	1.45
13	2.66	Cyclotrisiloxane	1.41
14	17.40	3-quinolinecarboxylic acid	1.31
15	6.17	Bicyclo[2.2.1]heptane, 7,7-dimethyl-1-2-methylene	1.23
16	12.69	1-octadecene	1.20
17	14.97	1-bromo-11-iodoundecane	1.18
18	11.49	Nonadecane	1.11
19	14.68	Kolavenol acetate	1.09
20	13.75	14-beta-H-pregna	1.07
21	10.10	6-hydroxycariophyllene	1.03



Figure 3. Preparation of O/W nanoemulsion of merbau extract and nutmeg leaf essential oil.

Particle Size Test, Polydispersity Index, and Bioactivities of Nanoemulsion

Particle size assessments were conducted to verify that the generated formulations fulfilled the dimensional and homogeneity criteria required for nanoemulsions, with the outcomes detailed in Table 6.

Data are presented as mean $\pm$ standard deviation (SD). The experimental unit and replicate structure were defined as follows: $n = 3$; experimental

replicates. Technical replicates were averaged before inferential analysis so that the independent preparation remained the experimental unit. Differences among F1, F2, and F3 were evaluated using one-way analysis of variance after assessment of normality and homogeneity procedures, if actually performed. When the overall ANOVA was significant, Duncan's multiple range test was applied at $\alpha = 0.05$. Exact p-values are reported, except values below 0.001, which are reported as $p < 0.001$. Analyses were conducted using IBM SPSS Statistics.



Figure 4. Microscopic appearance of the O/W nanoemulsions; (a) F1; (b) F2; (c) F3 (observed under a light microscope at 400× magnification).

Table 6. Characteristics, antioxidant and antityrosinase activity of nanoemulsion

Formula	Particle Size (nm)	Index Polydispersity	Antioxidant Activity (ppm)	Antityrosinase Activity (ppm)
F1	57.6	0.232	56.67 ± 0.57 ^a	154.67 ± 1.55 ^a
F2	531.3	0.358	21.64 ± 0.10 ^b	17.15 ± 0.08 ^b
F3	590.4	0.421	19.40 ± 0.48 ^c	11.43 ± 0.28 ^c

Data are presented as mean ± SD; n = [3, experimental replicates]. Different letters indicate significant differences based on DMRT ($p < 0.001$)

Data from Table 6 reveals a positive correlation: an increase in active ingredient concentration leads to a corresponding enlargement in particle size. While Formula 1 (57.6 nm) falls strictly within the conventional < 100 nm nanoparticle threshold, Formula 2 (531.3 nm) and Formula 3 (590.4 nm) expand into the sub-micron emulsion range. In dermatological delivery systems, lipid-based nanoemulsions and sub-micron emulsions are widely accepted to encompass droplet diameters up to 500–600 nm [31]. This dimensional expansion is a physical trade-off for encapsulating an exceptionally high active payload (30% extract in F3). This phenomenon is consistent with previous findings indicating that elevating the concentration of agents like chitosan, while keeping crosslinkers such as sodium tripolyphosphate (NaTPP) constant, triggers particle expansion due to agglomeration tendencies [32]. Forcing the F3 droplets below 100 nm at a 30% active load would require an excessive concentration of synthetic surfactants, which is undesirable for natural cosmetic applications.

Furthermore, despite the larger size in F3, the polydispersity index (PDI) for all formulations was recorded below 0.5, satisfying the standard requirement for high-quality nanoemulsions. Lower PDI values directly correlate with enhanced uniformity in droplet size distribution and lack of coalescence [33]. In comparative terms, Formula 1 exhibited the most optimal particle size, whereas Formula 3

demonstrated superior biological activity. Statistical evaluation at a 95% confidence interval confirmed that the active ingredient concentration significantly impacted the antioxidant capacity. Duncan's multiple range test identified the strongest antioxidant potential in F3 (19.40 ppm), followed by F2 (21.64 ppm), with F1 presenting the lowest activity (56.67 ppm) (Table 6). This trend aligns with the principle that higher concentrations of active compounds directly enhance antioxidant efficacy [34]. Similarly, the antityrosinase assay yielded IC_{50} values of 154.67 ppm for F1, 17.15 ppm for F2, and 11.43 ppm for F3, establishing F3 as the best overall formulation.

The formulated systems showed higher IC_{50} values than the crude merbau extract, indicating weaker apparent DPPH radical-scavenging and tyrosinase-inhibitory activities under the assay conditions. This difference may partly reflect the use of total-formulation concentration rather than merbau-extract-equivalent concentration. In addition, partitioning of the active compounds within the dispersed phase, restricted interaction with the DPPH radical or enzyme–substrate system, matrix colour/turbidity, excipient effects, and processing-related degradation may influence the measured response. Because encapsulation efficiency and release kinetics were not evaluated, the present results do not demonstrate controlled release or improved bioavailability. Future studies should compare activities on a merbau-extract-equivalent

basis and include merbau-only, essential-oil-only, and blank-formulation controls.

Ultrasonication is a preferred method for laboratory and pilot-scale nanoemulsion synthesis. The process leverages acoustic cavitation to generate localized turbulence and extreme pressure, effectively shearing oil droplets into the sub-micron or nano-range to bolster emulsion stability. While extensive literature reports droplet dimensions between 100 and 200 nm utilizing ultrasonic techniques, industrial scale-up remains a significant hurdle. Transitioning to large-scale production often mandates precise modifications to operating parameters, surfactant systems, or the core homogenization technology [17]. While Formula 3 demonstrated optimal physicochemical stability and promising *in vitro* antityrosinase activity, definitive safety evaluations—such as cytotoxicity assays, cell viability, and skin irritation testing were not conducted in this phase. This absence represents a limitation of the current preliminary study. Nevertheless, the formulation relies strictly on well-established cosmetic excipients, including glyceryl caprylate, propylene glycol, glycerin, and Tween 80, all of which possess extensively documented dermatological safety and biocompatibility profiles. Comprehensive preclinical toxicological assessments and *in vivo* irritation testing will be prioritized in future investigations prior to clinical or commercial application

CONCLUSION

The results of this study demonstrate that merbau wood extract contains several predominant constituents, namely robidanol, artemidiol, sakuranetin, sayanedin, and 4-methyl-6,7-dimethoxycoumarin, as identified by LC–MS analysis. In comparison, GC–MS analysis of nutmeg (*Myristica fragrans*) leaf essential oil revealed the presence of major compounds including myristicine, cyclotrisiloxane, 2-pinene, 1,4-cyclohexadiene, and 1,3-benzodioxole. Regarding bioactivity, the merbau extract exhibited strong antioxidant potential with an IC_{50} value of 11.25 ppm, whereas the nutmeg leaf essential oil showed markedly weaker antioxidant activity, with an IC_{50} exceeding 1000 ppm. Furthermore, the merbau extract demonstrated significant antityrosinase activity, indicated by an IC_{50} value of 6.82 ppm. Among the tested nanoemulsions, formulation 3, containing 30% active ingredient, was identified as the optimal formulation. This formulation produced a nanoemulsion with an average particle size of 590.4 nm and exhibited antioxidant activity with an IC_{50} of 19.40 ppm, as well as antityrosinase activity with an IC_{50} of 11.43 ppm.

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