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Nanoherbal from Ethanol Extract of Dutch Teak Leaves (*Guazuma ulmifolia* Lamk.) and Antibacterial Potential Against *Echerichia coli*

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Abstract: *Guazuma ulmifolia* Lamk. leaves, commonly known as Dutch teak leaves, are a medicinal plant traditionally used to treat diarrhea, infections, obesity, diabetes, and hypertension. Dutch teak leaves contain secondary metabolite compounds, including alkaloids, flavonoids, saponins, steroids, triterpenoids, tannins, phenolics, and glycosides. The presence of these compounds suggests that Dutch teak leaves have potential antibacterial activity. Herbal extracts have a limitation: low bioavailability. Therefore, nanoherbal formulations are used to improve their bioavailability. Nanoherbal is a nanoparticle made from plants. Nanoherbal can increase the surface area of medicinal herbs, thereby enhancing the solubility of compounds in the body. This study aims to determine the characteristics of nanoherbal derived from the ethanol extract of Dutch teak leaves and its antibacterial activity against Escherichia coli. The nanoherbal was synthesized using the ionic gelation method, employing alginate as the polyelectrolyte polymer and calcium chloride ($CaCl_2$) as the crosslinking agent. Four $CaCl_2$ concentrations were used: F1 (0.005%), F2 (0.01%), F3 (0.02%), and F4 (0.03%). Nanoherbal characterization, including particle size, zeta potential, and functional groups. The optimal nanoherbal formulation (F4) exhibited a particle size of 314.06 nm, a polydispersity index (PDI) of 0.0990, and a zeta potential of -131 mV. The FTIR spectra of nanoherbal showed a decrease in transmittance in the O-H, symmetric, and asymmetric COO- bands. The antibacterial activity was evaluated using the disc diffusion method. All treatments were conducted in triplicate, and results were expressed as average inhibition zone diameters (mm) and analyzed descriptively. The antibacterial activity test showed that the synthesized nanoherbal exhibited very strong activity against *Escherichia coli*, with an inhibition zone diameter of 23.65 mm. The synthesized nanoherbal showed a larger inhibition zone compared to the extract and alginate.

Keywords: Antibacterial Activity; Dutch Teak Leaves; Ionic Gelation; Nanoherbal.

Introduction

Dutch teak plant (*Guazuma ulmifolia* Lamk.) is a tropical plant indigenous to Latin America, particularly Brazil and Mexico. In its native regions, it is commonly referred to as *Guácimo* or *Mutamba*. Apart from its growth in these regions, this plant is also distributed in Panama, Ecuador, and several other countries in Latin America [1]. This plant is widely found in Indonesia, particularly on Java and Sumatra. The Dutch teak leaves are well known among the general public as a traditional remedy for obesity [2]. Moreover, Dutch teak leaves are traditionally used to treat various conditions, including skin diseases, asthma, fever, kidney, and liver disorders [3]. The secondary metabolite compounds content in Dutch teak leaves, including alkaloid, flavonoid, saponin, steroid, triterpenoid, tannin, phenolic, and glycoside [4]. In terms of pharmacology, Dutch teak leaves have antibacterial, antioxidant, and antidiarrheal activities [5].

Extract formulation in drug delivery has limitations, such as being difficult to dissolve in water, which results in low bioavailability in the body [6]. This limitation can be overcome by formulating the extract into a nanoherbal. Nanoherbal is nanoparticles derived from plants. In nanoherbal, active compounds in plants will be encapsulated within nanoparticle structures ranging in size from 1 to 1,000 nm [7]. Particle size is significant in drug delivery systems. Nanoherbal particles with small particle sizes will have a large surface area, enhancing electrostatic interactions between the bacterial cell surface and the active compounds. This increased interaction results in a stronger antibacterial effect. Furthermore, the greater surface area allows nanoherbal to adhere extensively to bacterial cells. This results in instability of the bacterial cell membrane, causing leakage of intracellular components, leading to bacterial lysis [8]. Beyond antibacterial mechanisms, nanoherbal also offers advantages in drug delivery systems, including capable to target active compounds to specific locations in the body, providing protection for active substances against environmental degradation, and reducing the likelihood of irritation to the digestive system [7], [9].

In this study, the nanoherbal was synthesized by the ionic gelation method. Ionic gelation is a nanoherbal synthesis approach that relies on electrostatic attraction between oppositely charged ions, typically requiring the presence of at least one polymer [10]. The polymer used in this study is sodium alginate. Sodium alginate is a polysaccharide derived from brown algae (*Phaeophyceae*). Alginate has good biocompatibility, biodegradability, and non-toxicity [11]. However, alginate has low viscosity and

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solubility, and is unstable at low pH, so CaCl2 must be added as a crosslinking agent. $CaCl_2$ can increase alginate viscosity under physiological conditions and strengthen the mechanical and chemical resistance of the resulting matrix [12].

Several plant extracts have been formulated into a nanoformulation through the ionic gelation method, focusing primarily on synthesis and characterization, such as nanoherbal from the extract of *Imperata cylindrica* L. [12], *Clitoria ternatea* L. [13], and *Areca catechu* L. [14]. Some studies also reported enhanced biological activity after nanoformulation. Recent studies have reported that plant extract-based nanoformulations enhance antibacterial activity against *Escherichia coli* compared to crude extracts [9], [15]. However, to the best of our knowledge, ethanol extract of Dutch teak leaves has not been formulated into a nanoherbal nor evaluated for its antibacterial activity against *Escherichia coli*. Therefore, this study aims to synthesize and characterize a nanoherbal formulation from ethanol extract of Dutch teak leaves using the ionic gelation method and to evaluate its antibacterial activity against *Escherichia coli*.

Research Methods

The equipment used in this study included Particle Size Analyzer (PSA) (Biobase BK-802N), zetasizer nano (MAL1270823), Fourier Transform Infrared (FTIR) spectrophotometer (Thermo Scientific Nicolet iS10), rotary vacuum evaporator (Buchi R-215), centrifuge (Eppendorf Centrifuge 5810), autoclave (Tomy SX-500), incubator (Isuzu model 2-2195), and freeze dryer.

The materials used in this study included Dutch teak leaves obtained from UPT Materia Medica Batu, technical ethanol (96%) (PT. Chemindo Interbuana), alginate (Sigma Aldrich), sodium hydroxide (NaOH) (Merck), aquades (PT. Chemindo Interbuana), $CaCl_2$ (Merck), ethanol p.a (PT. Chemindo Interbuana), tween 80 (Merck), Mueller Hinton Agar (MHA) media (Merck), *ciprofloxacin* (PT. Hexpharm Jaya Laboratories), and *Escherichia coli* bacteria from Institute of Tropical Disease (ITD), Universitas Airlangga.

Dutch Teak Leaves Extraction

Dried Dutch teak leaves were extracted by maceration. A total of 500 g of dried plant material was soaked in 2,000 mL of 96% ethanol for 24 hours in a sealed container. The sample was then filtered to separate the filtrate from the residue. The obtained filtrate was concentrated using a rotary vacuum evaporator at 30°C to produce a thick extract, which was subsequently stored at 4°C [12].

Nanoherbal Synthesis and Characterization

In this study, various formulations were prepared as presented in Table 1, with a total volume of 350 mL. Sodium alginate solution (0.1% w/v) was prepared by weighing 0.1 g of alginate and dissolving it in 100 mL of 0.1 M NaOH, under magnetic stirring until completely dissolved. A total of 1 g of thick extract was dissolved in 35 mL of ethanol p.a, and mixed with 15 mL of aqueous solution. Afterwards, 100 mL of 0.1% (w/v) sodium alginate solution was added, followed by the dropwise addition of 2 mL of Tween 80. The resulting solution mixture was gradually added to the various $CaCl_2$ formulations (0.005%-0.03% w/v). The solution was continuously stirred for 2 hours at 1500 rpm using a magnetic stirrer. The obtained nanoherbals were characterized for particle size and zeta potential using PSA and Zetasizer Nano. The solution mixture was centrifuged at 8000 rpm for 60 minutes. The residue was washed with aqueous and then freeze-dried for 16 hours to obtain a solid product. The solid was then used for functional group analysis using FTIR [16], [17], [18].

Table 1. Nanoherbal formulation

Materials	Formulations (% w/v)			
	F1	F2	F3	F4
Alginate	0.1 %	0.1 %	0.1 %	0.1 %
$CaCl_2$	0.005%	0.01%	0.02%	0.03%

Antibacterial Activity Test

This study was conducted using a completely randomized design, and each treatment was performed in triplicate. This study evaluates the antibacterial activity of nanoherbal formulations from the ethanol extract of Dutch teak leaves and alginate. Ciprofloxacin was used as a positive control, while aquades was used as a negative control. The antibacterial activity test began with the sterilization of tools and materials, followed by the preparation of MHA media. *Escherichia coli* bacteria suspension was prepared and adjusted to a 0.5 McFarland standard. Antibacterial testing was conducted by immersing a 6 mm diameter disc paper in the test solution, then placing it on MHA media and incubating for 24 hours at 37°C. Antibacterial activity was determined by measuring the diameter of the clear zone formed around the paper disc. The research results were analyzed descriptively using quantitative methods to determine the antibacterial activity based on the measured diameter of the inhibition zone.

Results and Discussion

Dutch Teak Leaves Extraction

Dutch teak leaves were extracted using the maceration method with 96% ethanol as the solvent. The advantage of this method is that the extraction process does not require high temperatures, thereby preventing degradation of compounds in the sample [19]. Ethanol 96% is used as a solvent because it is non-toxic, more selective, and able to attract polar, semipolar, and non-polar compounds from the sample [20]. The result is a thick, blackish-green extract weighing 58.49 grams, yielding a value of 11.70%.

Nanoherbal Synthesis and Characterization

Nanoherbal from Dutch teak leaves were synthesized using the ionic gelation method, employing alginate as polymer and $CaCl_2$ as crosslinking agent. During synthesis, the COO^- group of alginate interacts with divalent ions, such as Ca^{2+}, to form a three-dimensional structure that can encapsulate medicinal herbs [21]. The results of nanoherbal

synthesis are presented in the form of dark green colloids, as shown in Figure 1.

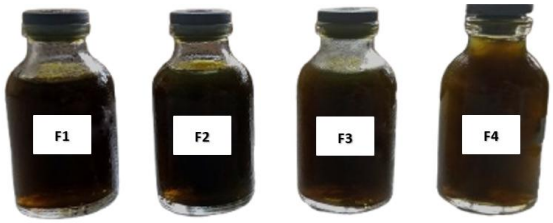

Figure 1. Nanoherbal from the ethanol extract of Dutch teak leaves

Particle size characterization of the nanoherbal colloid was performed using PSA. The results are presented in Table 2, showing that all nanoherbal formulations fall within the nanoscale size range of 1–1,000 nm. Therefore, all four formulations (F1–F4) remain within the nanoscale range and can be classified as nanoherbal formulations [7]. Nanoherbal (F4) has the smallest particle size of 314.06 nm, making it the optimum nanoherbal formulation. Smaller particle sizes enable easier cellular penetration, which in turn increases absorption in the body [9]. The optimal nanoherbal formulation was subjected to further characterization, including zeta potential measurement and functional group analysis.

Table 2. Particle size of nanoherbal synthesis

Nanoherbal Formulation	Alginate (%)	$CaCl_2$ (%)	Particle Size (nm)	PDI Value
F1	0.1	0.005	416.69	0.1821
F2	0.1	0.01	507.27	0.4889
F3	0.1	0.02	332.64	0.2148
F4	0.1	0.03	314.06	0.0990

The polydispersity index (PDI) value of nanoherbal (F4) is 0.0990. The PDI value approaching 0 indicates that the particles are increasingly homogeneous and stable. A PDI value >0.5 or approaching 1 indicates that the particles are heterogeneous or have low homogeneity [22]. Thus, the nanoherbal particles (F4) are distributed homogeneously. In this study, tween 80 was used as a surfactant that plays a role in producing stable nanoparticles and preventing agglomeration between particles [23].

The zeta potential was characterized using a Zetasizer Nano. Zeta potential refers to the electrical charge present on the surface of nanoparticles. Electric charge is closely related to electrostatic repulsion between particles. A good zeta potential value is > +30 mV and < -30 mV. Nanoherbal (F4) has a zeta potential of -131 mV, indicating high stability. Nanoherbal with a high zeta potential value shows a strong attractive force between particles, so that the stability of the emulsion is better, which can prevent aggregation between particles [24].

FTIR spectra of alginate, nanoherbal (F4), and ethanol extracts of Dutch teak leaves are shown in Figure 2. The interaction between alginate and the extract can be observed from the decrease in the number of waves in the O-H and COO^- functional groups [25]. However, in this study, the decrease in the number of wavelengths did not occur significantly, so that the interaction could be observed from the decrease in the transmittance value (%T) [26]. A decrease in transmittance values occurred in the O-H functional group, from 61.94% (alginate) to 11.92% (nanoherbal), indicating an interaction between the hydroxyl group in the active compound and the carboxylate group (COO^-) in the alginate matrix. In addition, there was a decrease in transmittance for the COO asymmetric functional group, from 76.06% (alginate) to 49.60% (nanoherbal). A decrease in transmittance values was also observed in the symmetrical COO^- functional group, from 75.34% (alginate) to 58.88% (nanoherbal). This indicates the formation of an interaction between the carboxylate group (COO^-) in the alginate matrix and the active compounds in the extract through hydrogen interaction [27].

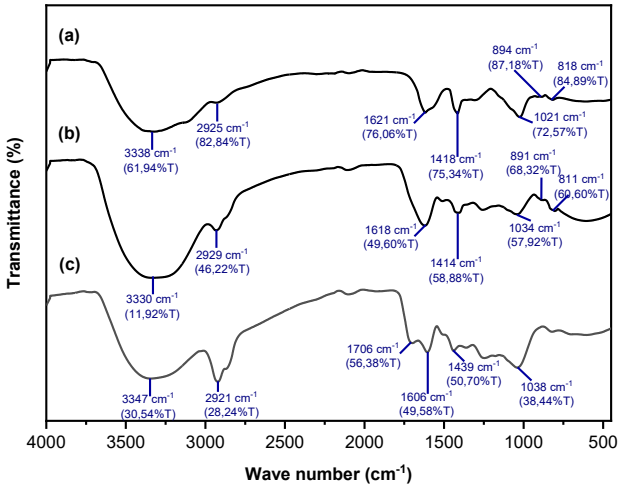

Figure 2. FTIR spectra (a) Alginate, (b) Nanoherbal (F4), (c) Ethanol extract of Dutch teak leaves

Antibacterial Activity Test

The antibacterial activity test aims to determine the effectiveness of nanoherbal in inhibiting the growth of *Escherichia coli.* The results of the antibacterial activity test are shown in Figure 3. The inhibition zone diameter data are presented in Table 3.

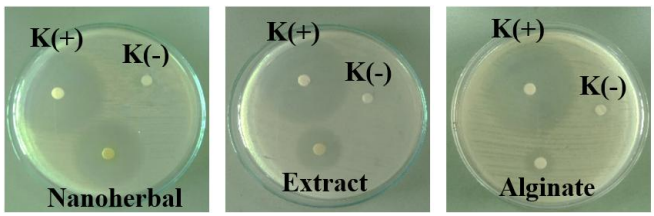

Figure 3. Antibacterial activity test

The nanoherbal formulation (F4) exhibited stronger antibacterial activity compared to the ethanol extract of Dutch teak leaves and alginate. Thus, nanoherbal formulation (F4) exhibits potential for further development as an antibacterial agent. The content of secondary metabolite compounds, including alkaloids, flavonoids, saponins, triterpenoids, and phenolics, plays a significant role in its antibacterial activity. Alkaloids act as antibacterials by inhibiting the synthesis of peptidoglycan, which causes damage to the structure of bacterial cell walls [28]. Flavonoids act as antibacterial agents by forming complexes with extracellular and dissolved proteins, thus causing damage to the structure of cell membranes [29].

Saponins inhibit bacterial growth by disrupting cell membrane permeability, leading to the leakage of essential cellular components [30]. Triterpenoids disrupt the permeability of bacterial cell membranes, leading to cellular imbalance [31]. Phenolic compounds can damage bacterial cell walls through enzyme inactivation and protein denaturation mechanisms [32].

Table 3. Inhibition zone diameter

Sample	Inhibiiton zone diameter (mm)				Category
	1	2	3	Average	
Nanoherbal (F4)	24.25	21.84	24.85	23.65	Very strong
Ethanol extract of Dutch teak leaves	14.34	18.54	16.03	16.30	Strong
Alginate	8.92	8.05	5.99	7.65	Medium
Positive control (Nanoherbal (F4))	30.20	32.67	33.78	32.22	Very strong
Positive control (Ethanol extract of Dutch teak leaves)	35.30	37.12	35.13	35.85	Very strong
Positive control (Alginate)	34.02	33.43	33.07	33.51	Very strong
Negative control (Aquades)	-	-	-	-	-

Nanoherbal (F4) shows greater antibacterial activity compared to the extract and alginate. This enhanced effect may be associated with its relatively smaller particle size (314.06 nm), which provides a larger surface area for interaction with bacterial cell membranes [33]. Increased surface contact facilities closer interaction between active compounds and negatively charged bacterial surface, potentially leading to membrane destabilisation. The nano formulation improves the efficiency of compound delivery and interaction by increasing the contact area. This enhanced interaction may increase membrane permeability, resulting in leakage of intracellular components and subsequent bacterial cell damage [8]. In addition, alginate, as a polyelectrolyte, also acts as an antibacterial agent by inhibiting the growth of the cytoplasmic membrane, allowing the intracellular components of the bacteria to exit and the bacteria to lyse [34].

Conclusion

This study successfully formulated a nanoherbal from the ethanol extract of Dutch teak leaves. The optimized formulation (F4) exhibited a particle size of 314.06 nm, a polydispersity index of 0.0990 and a highly negative zeta potential of -131 mV, which indicates good colloidal stability. FTIR analysis confirmed the successful formation of the nanoherbal system through changes in characteristic functional group transmittance. Nanoherbal (F4) exhibited very strong antibacterial activity against Escherichia coli. The antibacterial activity shown by nanoherbal (F4) was stronger compared to the ethanol extract of Dutch teak leaves. These findings suggest that nanoherbal formulation has potential for further development as a plant-based antibacterial delivery system. Future studies should include cytotoxicity evaluation, in vivo antibacterial assessment, and further optimization of formulation parameters to improve stability and therapeutic efficacy.

Author's Contribution

W.D. Larasati: Collect data and compile the article; S. Sutoyo: Responsible person and article compiler; Radita Yuniar Arizandy and Wahyu Setyarini: Guided and assisted in antibacterial activity testing.

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