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Phytochemical Screening and Nanoherbs Synthesis of Ethanol Extract of the Butterfly Pea Flower (*Clitoria ternatea* L.) with its Characterization

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Abstract: Every plant has a different secondary metabolite content, influenced by the location and conditions where the plant grows. Preparations from the butterfly pea flower (*Clitoria ternatea* L.) extract form have poor bioavailability. One effort to overcome this problem is by formulating it into nanoherbs form. This research aims to determine the phytochemical content and synthesize nanoherbs ethanol extract of butterfly pea flowers. The butterfly pea flowers were extracted using the maceration method, and the resulting thick purplish blue extract was 145.254 grams. The thick extract was analyzed for secondary metabolite content using phytochemical screening, and it was found to contain alkaloids, flavonoids, saponins, triterpenoids, and tannins. Nanoherb synthesis uses the ionic gelation method with an alginate polymer and a $CaCl_2$ cross-linking agent. The synthesis result is a purple nanoherbs colloid with optimal variations in the ratio (10:1). The synthesized nanoherbs were characterized using PSA and obtained particle size results from the optimal variation of 220.4 nm, polydispersity index 0.2550, and zeta potential of -22.5 mV.

Keywords: Alginate; Butterfly Pea Flower; Ionic Gelation; Nanoherbs Phytochemical.

Introduction

Plants can be used as herbal medicines to treat a disease. This is because medicinal plants contain secondary metabolite compounds that can have biological activity [1]. Several benefits of using plants as herbal medicines, including the raw materials used, are easy to obtain, medicinal plants can be grown in the home environment, they have affordable prices, they are relatively safe to use, and the side effects caused are also low [2] One type of medicinal plant commonly used is butterfly pea flower (*Clitoria ternatea* L.). Butterfly pea flowers are legumes and belong to the *Fabacea*e family. Butterfly pea flowers have various benefits for the body. These benefits include antioxidants, antibacterials, anticancer, anticholesterol, antidiabetes, anti-inflammatory, anthelmintic or antiparasitic drugs, fever, and pain relievers [3].

The community uses Butterfly pea flowers as medicinal plants because these plants contain various kinds of secondary metabolite compounds such as alkaloids, flavonoids, and saponins [3,4]. However, each plant has a different content of secondary metabolite compounds, influenced by the location and conditions of the place where the plant grows [5]. This research used butterfly pea flower plants, which were obtained from different places with previous studies.

Most active compounds in butterfly pea flowers have low bioavailability due to the low solubility of compounds in water and poor absorption. The large particle size of the compound also causes active substances to have difficulty penetrating the lipid membrane of body cells [6]. One of the efforts that can be applied to overcome the constraints of the active compounds in butterfly pea flowers is nanoparticle technology. Nanoparticle systems can protect and maintain an active substance's stability through encapsulation in a nanoparticle matrix [7,8].

Nanoparticles are colloidal particles with a size range of 10-1000 nm [9]. Nanoparticles derived from herbs are called nanoherbs [10]. Nanoparticle size can deliver drugs to target cells, increase surface area so that the solubility becomes high, and increase absorption in the small intestine to improve bioavailability, which is not good. Nanoparticles can increase mass transfer, increasing drug absorption and effectiveness[11,12].

Some of the advantages of nanotechnology in herbal medicine include overcoming the solubility of an insoluble active substance, improving poor bioavailability, and modifying the delivery system of the drug's active ingredients. Therefore, the active ingredients of drugs can go directly to specific areas such as cancer cells, red blood cells, and the digestive system. In addition, nanoparticles can also be used to increase the stability of active substances due to environmental degradation, such as enzymatic decomposition, hydrolysis, and oxidation, and reduce the irritating effects of active substances that occur in the digestive tract [7,9,13].

Nanoherbs can be prepared using various methods such as the reverse micelle method, emulsification, and cross-linking, phase inversion precipitation, droplet-emulsion coalescence, ionic gelation, ionic gelation with radical polymerization, self-assembly, top-down and spray drying [14]. The ionic gelation method is a method that involves a cross-linking process between polyelectrolytes in the presence of their multivalent ion pairs. Ionic gelation is followed by complexation of the polyelectrolyte with the opposite polyelectrolyte. The mechanical strength of the particles that will be formed will be stronger due to the formation of cross-linking bonds [15]. The advantages of this

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method include being easy to do, the materials used being easy to obtain, requiring relatively little solvent, not requiring expensive costs, and having good biocompatibility properties [16].

Cross-link nanoparticles can be made through cross-linking between electrolytes and their ion pairs. This bond occurs ionically or covalently. Cross-linked nanoparticles can be made using the ionic gelation method because there is no excessive stirring, it does not use high heat, and it avoids using organic solvents [15]. The principle of the ionic gelation method is based on the electrostatic interaction of groups with positive and negative charges that will form bonds with each other and increase the mechanical strength of the particles to be formed [17]. The ionic gelation method in cross-linking sodium alginate with $CaCl_2$ will form a more stable nanoparticle preparation [18].

Alginate, an anionic polymer, can be ionically cross-linked into an aqueous solution when added with divalent cations such as CaCl2[19]. Alginate is a linear polysaccharide derived from cell walls and the main component of brown seaweed sap. Alginate is a pure polymer of uronic acid, namely manuronic acid and guluronic acid, connected through 1,4 bonds in the form of long linear chains and arranged in homogeneous or heterogeneous block patterns. Alginate has properties that are biocompatible, biodegradable, and non-toxic when entering the body [16]. Sodium alginate has the physical characteristics of flour or fiber: white or yellowish, almost odourless, and tasteless [20].

In this research, phytochemical screening and nanoherbs synthesis of ethanol extract of butterfly pea flower has not been conducted. In addition, nanoherbs characterization is also carried out using the PSA (Particle Size Analyzer) instrument to determine the particle size and zeta potential.

Research Methods

The method in this research is experimental research. The equipment used in this research is a set of maceration tools, a Particle Size Analyzer (Microtrac), Scanning Electron Microscopy (Hitachi S-4500), analytical balance (Adventurer Ohaus), hot plate, and magnetic stirrer (Thermo Scientific), centrifuged (Beckman Coulter Allegra 64R) rotary vacuum evaporator (Buchi R-300), vacuum pump, glass tools, volume pipettes, vials, filter paper, and Buchner funnel.

The materials used in this research are butterfly pea flowers, ethanol p.a. Merck, 96% technical ethanol, alginate, calcium chloride ($CaCl_2$), distilled water, Dragendorf reagent, Mayer reagent, Wagner reagent, chloroform, ammonia, 2N sulfuric acid, 70% ethanol, Mg powder, concentrated HCl, concentrated sulfuric acid, anhydrous acetic acid, and $FeCl_3$ 1%.

This study begins with the extraction of butterfly pea flowers. This extraction process uses the maceration method with 96% ethanol solvent [10]. After obtaining butterfly pea flower extract, it will be continued for phytochemical screening. First is an alkaloid test. A total of 1 mL of extract was mixed with 1 mL of chloroform and 1 mL of ammonia. Then heated, shaken, and filtered. The filtrate that has been obtained is added to 3 drops of 2N sulfuric acid. Then, the top of the filtrate was taken and tested using Dragendorf reagent, Mayer reagent, and Wagner reagent [21]. Flavonoid testing methods involve extracting as much as 1 mL added with 3 mL of 70% ethanol and then shaking it. After that, it was heated in a water bath, shaken again, and filtered. The filtrate that has been obtained is added with Mg powder and 2-4 drops of concentrated HCl [22].

For the saponin test, 1 mL of extract was added with 10 mL of distilled water and then heated in a water bath. Then, they were shaken and allowed to stand for 15 minutes [22]. Next are steroid and triterpenoid tests. A sample of 1 mL of extract was mixed with 2 mL of anhydrous acetic acid and 2 mL of concentrated sulfuric acid [23]. The last phytochemical test is tannin. A total of 1 mL of extract was heated in a water bath. Then, a few drops (2-3 drops) of FeCl3 1% [22] were added.

After conducting phytochemical testing followed by the synthesis of nanoherbs, in this research, 3 variations of the ratio of sodium alginate solution and calcium chloride solution were used, namely (10:1), (5:1), and (2.5:1). The preparation of nanoherbs begins with weighing 1 gram of ethanol extract of butterfly pea flower dissolved with 35 mL of ethanol p.a and 15 mL of distilled water. Furthermore, 0.1% sodium alginate solution, as much as 100 mL, was added and stirred using a magnetic stirrer. After that, calcium chloride ($CaCl_2$) solution (concentration 0.01%, 0.02%, and 0.03%) as much as 350 mL was added to the mixture little by little under the rotation of the magnetic stirrer. Stirring was carried out for 2 hours at a constant speed of 1200 rpm. Colloidal nanoparticles that have been formed were then tested using PSA (Particle Size Analyzer) [19].

Table 1. Nanoherbs Formulation Design

Material	Formulation 1	Formulation 2	Formulation 3
Butterfly pea flower ethanol extract	1 gram	1 gram	1 gram
Sodium Alginate	0.1%	0.1%	0.1%
Calcium chloride ($CaCl_2$)	0.01%	0.02%	0.03%

Results and Discussion

Sample Extraction

In this research, the extraction process of butterfly pea flower samples used the maceration method. Maceration is an extraction method by soaking the simplisia using a suitable solvent in a container or vessel that is tightly closed at room temperature [24]. This method was chosen because it has several advantages, such as being simple, easy to do, not requiring much equipment, and being carried out at room temperature so that thermolabile compounds are not lost or damaged [25]. During the maceration process, the walls and cell membranes of the plant will decompose, which causes a pressure difference between inside and outside the cell so that secondary metabolite compounds will dissolve along with the organic solvent [22].

The high yield indicates that the active compounds contained in the sample are also higher [26]. In this research, 96% ethanol solvent was used so that more active

compounds could be extracted. The maceration results were obtained as a thick purplish blue extract as much as 145.254 grams, so the yield was 29.0508%.

Phytochemical Screening

The results of the phytochemical test showed that the ethanol extract of butterfly pea flowers contained several secondary metabolite compounds in the form of alkaloids, flavonoids, saponins, triterpenoids, and tannins. The test results can be seen in Table 2.

Table 2. Phytochemical Test Results of Ethanol Extract of Butterfly Pea Flower

Secondary Metabolite Compound	Result	Description
Alkaloids	-	*Dragendorf*: no precipitate formed
	+	*Mayer*: white precipitate formed
	+	*Wagner*: formed reddish brown precipitate
Flavonoids	+	Red color
Saponins	+	Formed a stable foam
Steroids	-	Brownish colour
Triterpenoids	+	Brownish red colour between ethanol layers
Tannins	+	Greenish black colour

Information:
(+) = There are secondary metabolite compounds

The results of this test indicate that the ethanol extract of butterfly pea flowers contains alkaloid compounds characterized by the formation of white precipitates on the Mayer reagent and reddish-brown precipitates on the Wagner reagent. The precipitate is produced due to the formation of the potassium-alkaloid complex. Nitrogen atoms contained in alkaloids have free electron pairs that will form coordinate covalent bonds with K^+ ions in the alkaloid reagent [22].

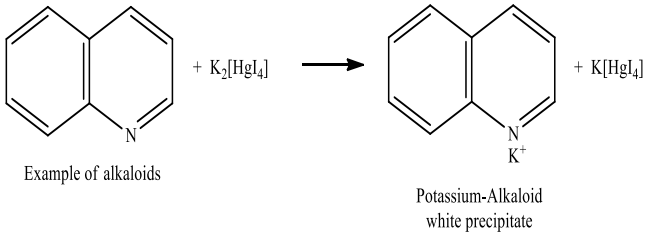

Figure 1. Mayer's Reaction [23]

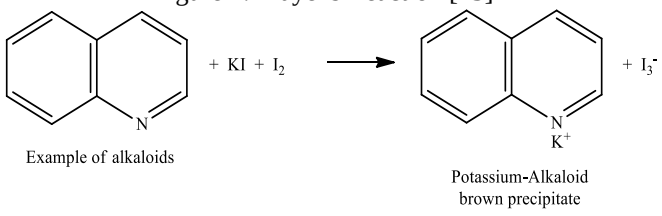

Figure 2. Wagner's Reaction [23]

The results of flavonoid testing show that the ethanol extract of butterfly pea flower is positive for flavonoids, characterized by forming a red solution. The formation of this red solution is due to the complex compound of Mg ions with phenoxy ions contained in flavonoid compounds. Polyhydroxy of flavonone will be reduced with Mg2+ ions in concentrated HCl to form benzopyrilium salts that are red, yellow, or flavylium salts [23].

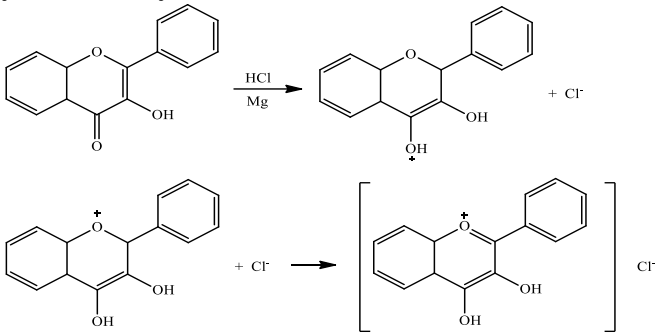

Figure 3. Reaction of Flavonoids with Magnesium [23]

In testing, saponin compounds contained in ethanol extract of butterfly pea flower showed positive results with the formation of stable foam for ± 15 minutes. Stable foam is due to glycosides that can obtain foam in water and then hydrolyze into glucose and other compounds [22].

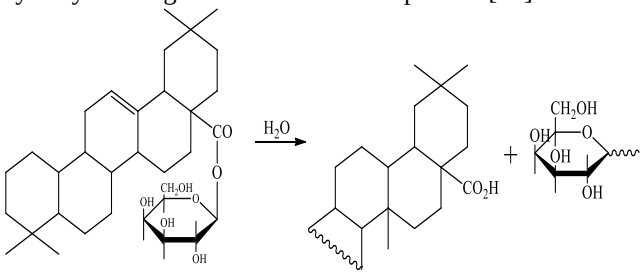

Figure 4. Saponin Test Reaction [23]

Testing for steroid compounds in ethanol extracts of butterfly pea flowers produces a brownish solution. This indicates that the ethanol extract of butterfly pea flowers does not contain steroid compounds. Positive steroid results are characterized by forming a bluish-green solution caused by steroid class compounds undergoing oxidation to form conjugated double bonds [22].

The positive results of triterpenoid compounds in this research indicate that the ethanol extract of butterfly pea flowers contains compounds of the triterpenoid group. A brownish-red colour is formed due to triterpenoid compounds forming colour by H_2SO_4 solution in anhydrous acetic acid. The colour difference formed due to steroid and triterpenoid compounds is due to the difference in groups on the C-4 atom [27].

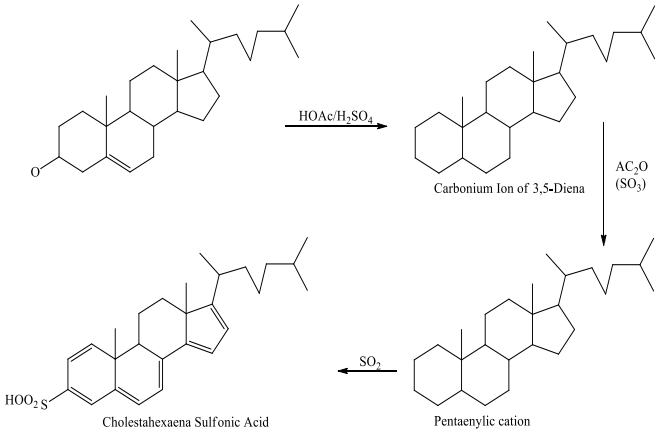

Figure 5. Steroid and Triterpenoid Testing Reaction [27]

Butterfly pea flower ethanol extract tested with $FeCl_3$ forms a green solution indicating that the extract contains tannin compounds. This green colour is due to the formation of tannin complex compounds with Fe^{3+} ions. This complex compound is formed due to the presence of Fe^{3+} ions as the central atom, and tannin has an O atom with a free electron pair that can coordinate with the central atom as a ligand [28].

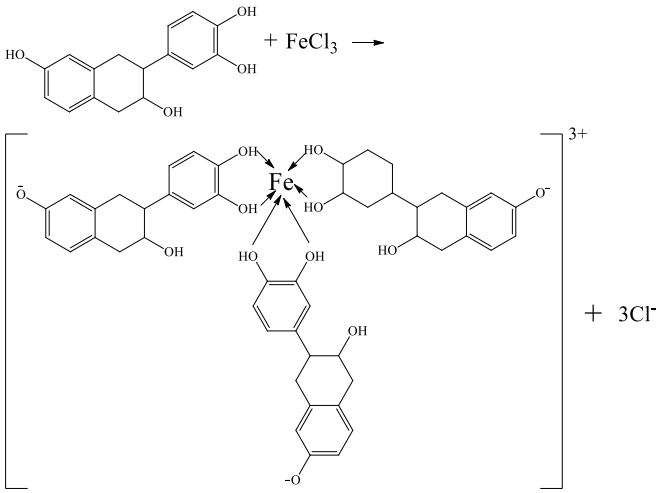

Figure 6. Reaction of Tannins with Polyphenols [22]

Nanoherbs Synthesis

Based on the procedure of making nanoherbs, the colloidal nanoherbs of ethanol extract from butterfly pea flowers were produced in purple. The method used in this research is the ionic gelation method because it is the easiest to do compared to other methods. The ionic gelation method involves cross-linking between the polyelectrolyte and its multivalent ion pair. The formed cross-linking can strengthen the particles' mechanical strength[29].

The cross-linking agent in $CaCl_2$ will interact with carboxylate groups derived from sodium alginate, replacing sodium ions in alginate with calcium ions, as shown in Figure 7 [30].

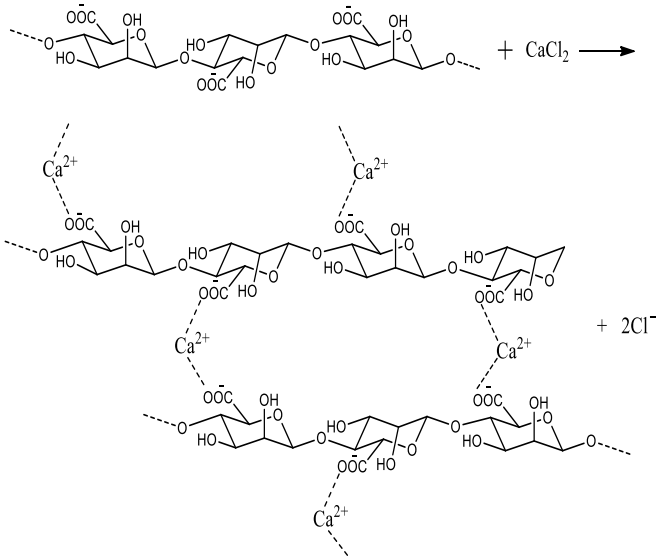

Figure 7. Cross-linking Na-alginate with Calcium Chloride [31]

Figure 8. Nanoherbs of Butterfly Pea Flower Ethanol Extract

Alginate is an anionic polymer that can react with divalent cations such as calcium chloride. The choice of alginate as a polymer in this research is due to its nature, which is biodegradable, biocompatible, and non-toxic when entering the body, so it is safe if applied as a medicine. When in the body, this polymer will experience swelling, which will then degrade and break up. Therefore, the secondary metabolite compounds from butterfly pea flowers absorbed in alginate will be released gradually in the body when applied as herbal medicine [19].

Characterization of Particle Size

The concentration of calcium chloride used in the preparation of nanoherbs can affect the particle size that will be formed. The higher the concentration of calcium chloride, the more bonds are formed between Ca2+ ions and carboxylic groups of alginate, resulting in the formation of soluble solids and causing the particle size to increase [32]. The results of particle size measurements of the three nanoherbs formulations can be seen in Table 3.

Table 3. Particle Size Measurement Results of Nanoherbs Ethanol Extract of Butterfly Pea Flower

Formulation	Particle Size (nm)	% Nano	PDI
1	220.4	100	0.2550
2	295.6	100	0.1936
3	302	92.2	0.1350

One of the most essential parameters in nanoherbs is determining particle size and particle size distribution. Based on the three formulations, all sizes can still be classified as nanoparticles. Nanoparticles are solid colloidal particles with a size range of 10-1000 nm [15]. The most optimal nanoherbs of the three formulations are in Formula 1. This is because the smallest particle size is 220.4 nm with a total percentage of 100% nanosize. The size is closest to the best size of nanoparticles used in herbal medicine in the range of 1-100 nm [33]. Nanoparticles with smaller sizes have a more excellent surface area-to-volume ratio, which allows for better absorption and increased bioavailability of herbal medicines [34].

The results of this research are supported by other research that has successfully synthesized nanoherbs using alginate polymers with the ionic gelation method. It is known that nanoherbs of *Kaempferia rotunda* extract has a particle size of 100 nm [19]. Meanwhile, nanoherbs of *Boesenbergia pandurata* extract produced nanoparticles with a particle size of 339 nm [32].

The polydispersity index is used to describe the homogeneity of a colloidal solution. The PDI (Polydispersity Index) value range is 0-1, but a good PDI value is <0.5, indicating that the particles are homogeneous. The particles have high heterogeneity if the value is >0.5[35]. If the nanoparticles have a PDI value of 1, the size distribution will be extensive and contain large particles or aggregates that can undergo sedimentation [18]. PDI measurement aims to determine the uniformity of the particles. In this research, the PDI value obtained in the optimal formula was 0.2550, meaning that the particle distribution range is homogeneous.

Characterization of Zeta Potential

The value indicating the repulsive force between particles is known as zeta potential, which is used to predict the stability of colloidal solutions. Nanoparticles with a zeta potential value of (+/-) 30 mV have higher stability [18]. Nilai potensial zeta dari nanoherbs formula optimal yaitu -22,5 mV. The zeta potential value of the optimal nanoherbs formula is -22.5 mV. Therefore, the nanoherbs of bay flower extract are not stable enough because it has a zeta potential value below 30 mV, making it easier to experience aggregation.

Conclusion

Based on the research that has been done, it can be concluded that the ethanol extract of butterfly pea flowers contains secondary metabolite compounds of alkaloid, flavonoid, saponin, triterpenoid, and tannin groups. These secondary metabolite compounds can increase their bioavailability by being made into nanoparticles. Nanoherbs butterfly pea flower ethanol extract can be made using the ionic gelation method with the most optimum concentration ratio of sodium alginate and calcium chloride, which is 10:1 in the form of a percent (%) Na-alginate 0.1% and calcium chloride 0.01%. The optimal formula produced a particle size of 220.4 nm, as much as 100%, a polydispersity index of 0.2550, and a zeta potential of -22.5 mV.

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