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NANOEMULSION EXTRACT ETHANOL *Brucea javanica* (L) Merr. FORMULATION USING TWEEN 20 & TWEEN 80 AS EMULGATOR

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A B S T R A C T

Brucea javanica (L) Merr. has the potential to be developed as a topical application as a result of its high flavonoid content. However, advancements in pharmaceutical preparation are constrained by the chemicals' poor bioavailability when prepared conventionally. Nanoemulsion preparation has been an efficient method for enhancing topical preparation absorption. The previous studies show that the stable formulation of nanoemulsion depends on emulgator, especially tween. This study's objective is to create an ethanol extract of Brucea javanica (L) Merr. using tween 20 and tween 80 as emulsifying agent. A pseudo-ternary phase diagram was utilized to formulate the nanoemulsion formula. Physical characteristics such as particle size, polydispersity index, zeta potential, percent transmittance, drug load, viscosity, and pH were then analyzed for the nanoemulsion. At 15 sampling points, the formula tween 20 (surfactant):PEG 400 (cosurfactant) in a 1:1 ratio did not produce a clear preparation. In contrast, tween 80 with the same cosurfactant and ratio formed a formula that was transparent. The formula was then examined for its physical parameters, which included a particle size of 17.90.14 nm, a polydispersity index of 0.0970.012, a zeta potential of 84.63% transmittance, a drug load of 99.97%, a viscosity of 240 cp, and a pH of 7.194. According to the results, tween 80 is the most suited emulsifier for the production of Brucea javanica (L) Merr extract nanoemulsion. The study of physical attributes reveals that the nanoemulsion generated satisfies the criteria for nanoemulsions with desirable physical properties. Furthermore, the study provides data required for the development of a nanoemulsion of ethanol extract of Brucea javanica.

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INTRODUCTION

Brucea javanica (L.) Merr., the perennial herbaceous plant belonging to the Simaroubaceae family, can be found in a num (Ablat et al., 2014) of Indonesian forests. *B. javanica* is indicated as ananti-diabetic (Muliasari et al., 2017), anti-inflammatory (Amini et al., 2020), anti-tumor (Liu et al., 2009). The presence of flavonoid, which is a phenolic component, is responsible for the biological activity of *B. javanica* (Ablat et al., 2014). Based onRizkia et al., (2022) total phenolic compound in the extract 67,985±0,0968 mg GAE/g extract.

Previous research has revealed that a 1% concentration of *B. javanica* extract inhibits protein denaturation, indicating anti-inflammatory action (Almira et al., 2021). In addition, cream-formulated *B. javanica* extract has been shown to protect against UV-induced skin damage in mice (Amini et al., 2020). However, the bioavailability and stability of existing conventional preparations are inadequate. A suitable delivery system needs to be developed in order to tackle these issues. Nanoemulsion is among the promising dosage types.

Nanoemulsion is a transparent or translucent oil-and-water dispersion that is stabilized by molecules of surfactant and cosurfactant. Nanoemulsions are resistant to aggregation and creaming since their particle size is less than 100 nm (Wu et al., 2020). Compared to other medical preparations, nanoemulsion has the advantage of increasing transdermal permease when administered topically.

Stable nanocarriers depend greatly on the excipient emulsifier chosen. Thus, two distinct surfactants were employed in this study. Tween 20 is a nonionic polysorbate surfactant generated by the erhoxylation of sorbitan before to the introduction of lauric acid, whereas tween 80 is formed prior to addition of oleic acid. Tween emulsifier is commonly employed in cosmetic and medicinal formulations due to its relative stability and nontoxicity. The two emulsifiers have identical polar heads but distinct apolar tails, hence their HLB values are distinct (Gorjian et al., 2022). Therefore, the object of the present work was to formulate a nanoemulsion extract of *B. javanica*with two different nonionic surfactants.

METHODE

Material

B. javanica fruits were collected in Narmada, West Lombok, and NTB, tikus, etanol 96%, rat, ethanol 96%, gallic acid (Merck), Na_2CO_3 (Merck), FollinCiaocalteu (Merck), aquadest, extra virgin olive oil (EVOO), tween 20 (Vorgonamari), tween 80 (Vorgonamari), isopropyl alcohol (Merck), propylene glycol (The dow chemical company), PEG 400 (Alpha chem), glycerin (Vorgonamari), methyl paraben (Welgo chemical technology), propyl paraben (Welgo chemical technology), sodium dihydrogen phosphate (RRC), and sodium hydrogen (Pudak)

Extract Preparation

The fruit of *B. javanica*was harvested when ripe and has a purplish color. *B. javanica* fresh fruits have been cleaned and dried. The dried fruit was peeled to reveal the seeds, which were then crushed. The dried simplicials were sonicated for 35 minutes at a 2:5 ratio, which

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used 96% ethanol as solvent. To create a thick extract, the filtrate was collected and heated to 40°C in a rotary evaporator.

Total Phenolic content determination

The total phenolic content of the extract was evaluated by using the Follin-Ciocalteceau method. 300 μL sample extract was added to 1 ml of 1 mL reagent Follin-Ciocalteceau, and it was then stirred. The mixture was shaken again after adding 1.2 mL of 7.5% Na2CO3 solution. The incubation period was 11 minutes and it was measured at 650 nm. Gallic acid's standard curve was used to calculate the total phenolic content, which was then represented as gallic acid equivalents (GAE) mg/g of dry extract (Verawati et al., 2017).

Preparation of CarrierNanoemulsion

EVOO was chosen as the oil phase, whereas tween 20 and tween 80 were chosen as the surfactants. In this investigation, the cosurfactants utilized were PEG 400 and isopropyl alcohol. Ternary phase diagrams of surfactants, co-surfactants, and oils were developed to identify the nanoemulsion production zone.To obtain a mixture of surfactants and co-surfactants, the surfactants and co-surfactants were mixed according to the ratio and vortexed for one minute. The ratio of surfactant to co-surfactant in the resulting mixture is 1:1. The mixture is titrated with distilled water, and magnetic stirring is used for mixing. Next, the sample is assessed for transparency and phase separation.

Preparation of Nanoemulsion of extract B. javanica

EVOO and *B. javanica* extract were mixed in a vial and homogenized using a magnetic stirrer. Add the emulsifier and cosurfactant and mix until uniform. The mixture is titrated with distilled water using a micropipette while being continuously stirred with a magnetic stirrer. The mixture was then mixed with propyl paraben, methyl paraben, and glycerin until an emulsion was created.

Percent transmittance measurement

The nanoemulsion preparation of the ethanolic extract of *B. javanica* was placed in a cuvette, and the percent transmissionwas read at a wavelength of 650 nm. The blank used is aquadest (Juliantoni et al., 2020).

pH measurement

The pH value of the nanoemulsion formulation of *B. javanica* ethanol extract was determined using a pH meter. The pH meter electrode is put into the nanoemulsion, and the pH meter instrument is left for a period of time until a steady pH reading is achieved (Juliantoni et al., 2020).

Viscosity measurement

The nanoemulsion of *B. javanica* ethanol extracts has been tested to a viscosity test using a Brookfield viscometer at 27^{o}C with a shear speed of 25-125 rpm for 1 minute with spindle number 64 (Hajrin et al., 2021).

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Drug load determination

The determination of drug load was based on the equivalent of gallic acid. 300 µL of the nanoemulsion preparation was pipetted, followed by the addition of 1 mL of Follin-ciocalteucl and vigorous shaking. After allowing the solution to stand for three minutes, 1.2 mL of a 7.5% Na_2CO_3 solution was added, the mixture was agitated to ensure homogeneity, and the solution was incubated during operating time. The sample's absorbance is measured at the maximum wavelength. The drug load is determined using an equation (1) (Juliantoni et al., 2020).

$$Drug\ Load\ (\%) = \frac{Total\ Phenolic\ Compounds\ Supernatant\ (mg\ EAG)}{Total\ Phenolic\ Compounds\ Ekstrak\ (mg\ EAG)} x\ 100\% \qquad (1)$$

Globules size determination

The size of globules was determined using a particle size analyzer (Malvern). A globule size distribution curve was obtained by scanning 1.0 g of nanoemulsion of *B. javanica* seed ethanol extract diluted with 10 ml of distilled water and placed in a cuvette (Juliantoni et al., 2020).

RESULT AND DISCUSSION

The fruit of *B. javanica* was collected in the Narmada region of West Lombok, NTB. The 1 kilogram of wet fruit is dried and then peeled to obtain the seeds. Attained dried seeds weighing up to 112 grams. 100g of dry simplicia was sonicated for 35 minutes in 96% ethanol. Since sonication uses ultrasonic waves to break down the sample's cell walls, using a sonication-based approach can decrease the number of discoveries and the amount of time required. The solvent then enters the cell and extracts the plant's secondary metabolites. This can minimize the extraction time, hence decreasing the amount of harmful chemicals in the solvent (Ranjha et al., 2021). The 96% ethanol solvent was used because to its polarity, which allows it to attract more polyphenol chemicals in plants while remaining harmless to people. Additionally, it is readily available, inexpensive, and universal (ErmiHikmawanti et al., 2021).

A rotary evaporator was then utilized to concentrate the sonicated extract. The extract obtained from the processis yellow, odorless, and viscous with a weight of 5.142 grams and a yield percentage of 5.142%.The organoleptic results obtained were identical compared to the research conducted by Almira et al., (2021), with the exception that the yield percentage was lower in this study. This is due to variations in growing locations and harvesting times.

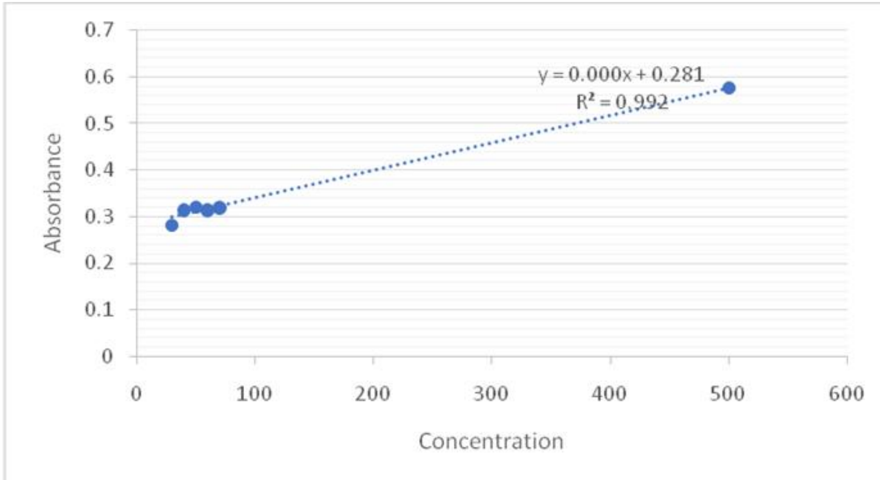

Figure 1. Galat acid calibration curve

As illustrated in Figure 1, the existing gallic acid measurement findings have been plotted on a curve. The connection between gallic acid content and absorbance is represented by the linear equation y = 0.0006x + 0.2813 with an R2 value of 0.9921. The closer the value of R2 is to 1, the better the linearity of the curve. This equation will be used to calculate the total phenolic content of the ethanol extract of *B. Javanica*. The total phenolic content of the *B. Javanica*extract was determined to be 140.6526 mg GAE/g of sample. This investigation yielded a higher phenolic value than the previous one, which yielded 49.46 mg GAE/g sample by maceration and 38.30 mg GAE/g sample by soxhletation (Risnadewi et al., 2019). This is due to sonication technique uses ultrasonic waves to break down the sample's cell walls, allowing solvents to more easily penetrate the cells and extract the plant's secondary metabolites (Ranjha et al., 2021). Therefore, the contact period between the solvent and the sample is significantly less than when employing the maceration technique. Long contact time can diminish the quality of the sample's metabolism. The sonication process does not employ high temperatures like the soxhletation process. Flavonoids and phenolics contained in a sample can be damaged by high temperatures. The flavonoid and phenolic chemicals found in the seeds of the *B. Javanica* are susceptible to high temperatures and can be destroyed (Ioannou et al., 2020).

The results of the nanoemulsion of *B. Javanica* extract using tween 20:PEG 400 in a 1:1 ratio show that no stable and transparent preparation appears. Meanwhile, the nanoemulsion of *B. Javanica* extract using tween 80:PEG 400 in a 1:1 only at point 3 shows transparance preparation. The result of the nanoemulsion of extract *B. Javanica* can be seen in Figure 2a. In addition, Figure 2b. depicts tween 80 and PEG 400 with a ratio of 1:1 using Pseudo-ternary phase diagrams.

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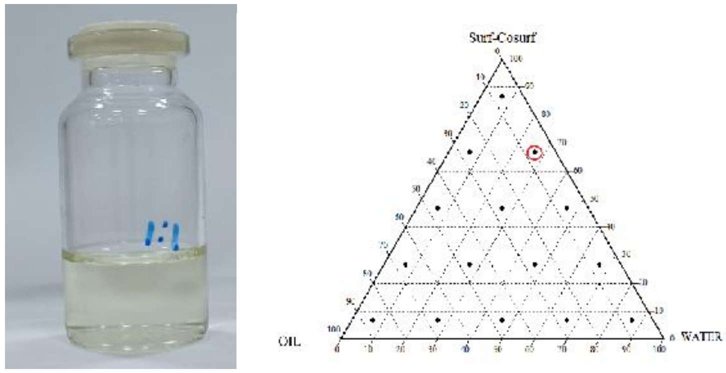

(a) (b)

Figure. 2(a) Nanoemulsion of *B. Javanica* extract tween 20:PEG 400 (1:1)

2(b)Pseudo-ternary diagram of *B. Javanica* extract Nanoemulsion

B. Javanica extract nanoemulsion formula. At point three, the formula consists of 66.7% surfactant, 6.7% oil, and 26.7% water. Nanoemulsion formation requires a surfactant value greater than 10 and is compatible with the utilized oil phase. The HLB tween 80 value is 15 and is compatible with extra virgin olive oil as the oil phase. In addition, the combination of co-surfactants plays an influence on the formation of nanoemulsions. Co-surfactants enhance the mobility of hydrocarbon tails, hence enhancing the penetration of monomer surfactants into the hydrophobic oil phase. This increase in penetration will contribute to lowering the surface tension, which will decrease the entropy system (Shaker et al., 2019).

Table 1. Physical characterization of Nanoemulsion of *B. Javanica* extract tween 80:PEG 400 (1:1)

Parameter	Result
Percent transmittance	84,63
pH	7,05
Drug load	99,97 %
Globul size	18,40±0,082 nm
Polydispersity index	0,089±0,013
Zeta Potensial	-0,87±0,04 mV

The percent transmittance indicates whether the nanoemulsion produced is transparent. A transmittance value nearing 100% indicates that the formed globules are nanoscale in size. Due to the nanoparticles' small size, light scattering will be prevented, and nearly all light will be transferred (Sundararajan et al., 2018). Table 1 displays the results of the percent transmittance test. It demonstrates that the average preparation yields a transmittance rate

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greater than 80%, as represented by the results of the clear preparation (Nandita et al., 2021). This shows that the globules of the preparation lie within the nanometer range.

The preparation's pH measurement is intended to produce preparations that are harmless to human skin. The preparation's pH value should lie within the physiological pH range of humans. The skin will become morescaly as the pH value of the product rises. On the other hand, as pH decreases, the risk of skin irritation and redness increases(Lukić et al., 2021). The results in Table 1 show that the nanoemulsion formula has a pH between 4.5 and 7.5.Moreover Table 1 displays the results of the drug load study. The nanoemulsion value exceeds 90%. The greater the drug load, the greater the quantity of extract dissolved in the preparation. The dissolved extract in the emulsion system is expanding.

According to Table 1, nanoemulsion lies within the nano range. The particle size is within the specified range of 1-100 nm. Another measure is the polydispersity index, which indicates the size-based heterogeneity of the sample. Polydispersity can be caused by the sample's size distribution or the agglomeration or aggregation of samples during the formulation process. The results indicated that nanoemulsion lay within the estimated region where the value is smaller than 1, thus classifying the system as a monodisperse system (Gurpret& Singh, 2018).The polydispersity index of nanoemulsion is 0,089±0,013; this value is greater than 0,7, indicating a broad droplet size distribution (Alhakamy et al., 2020). The formulation of nanoemulsion has a low zeta value. Because fatty acids are present, the zeta potential value is negative. The relationship between zeta potential and colloidal dispersion stability is substantial. The stable preparation has a zeta potential greater than 30mV. A low zeta potential value reduces interparticle repulsion, which facilitates particle flocculation (Zanela da Silva Marques et al., 2018).

CONCLUSION

According to the results, tween 80 is the most suited emulsifier for the production of *Brucea javanica* (L) Merr extract nanoemulsion. The study of physical attributes reveals that the nanoemulsion generated satisfies the criteria for nanoemulsions with desirable physical properties.

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