Microencapsulation of *Macaranga gigantea* Leaf Extracts: Production and Characterization

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**ABSTRACT**

**Introduction:** The aim of this research was to formulate the microcapsules of *Macaranga gigantea* leaves extract with solvent evaporation method using Ethocel 10 cP and Eudragit E100 as matrix. **Methods:** *M. gigantea* leaves were extracted using ethanol 96%. This extract was dried by rotary evaporator. The microencapsulation process of *M. gigantea* leaves extract was conducted by solvent evaporation method (IOW: oil in water). The formula of *M. gigantea* leaves extract microcapsules were designed into six formulas (Eudragit E100: FA1, FA2, FA3 and Ethocel 10 cP: FB1, FB2, FB3). Microcapsules of *M. gigantea* leaves extract were characterized for particle size, in terms of surface morphology by scanning electron microscope (SEM) and encapsulation efficiency. Antioxidant activity of the formulation have been evaluated by DPPH method. Physical characterization on microparticles were performed by conducting entrapment efficiency and SEM picture. **Results:** In this research, the microparticles containing *M. gigantea* extract has been developed by using ethyl cellulose (Ethocel 10 cP) and eudragit (Eudragit E100) as polymer matrix. The results showed that high concentration of polymer (Ethocel 10 cP and Eudragit E100) in microencapsulation resulted in better *M. gigantea* leaves extract microcapsules in terms of physical characteristics. Particle size of microcapsules containing *M. gigantea* leaves extract were in the range of 3.664 to 5.887 μm. Encapsulation efficiency (% EE) was categorized as good because the value were ≥ 80% to which 85.978% (FA3) and 88.992% (FB3). SEM picture of FA3 (Eudragit E100) revealed that the surface of microcapsule were rough and porous. When Ethocel 10 cP used as polymer, a smoother and less visible pores of microcapsule were obtained. The antioxidant ability of *M. gigantea* leaves extract microcapsule showed that IC50 values was 64.51 ppm. **Conclusion:** It can be concluded that microcapsules of *M. gigantea* leaves extract can be prepared by solvent evaporation method by using Eudragit E100 and Ethocel 10 cP as polymer matrix. *M. gigantea* leaves extract has potent antioxidant activity either as extract or after formulated into microcapsules.

**Key words:** Microencapsulation, Solvent evaporation method, *Macaranga gigantea*, Eudragit E100, Ethocel 10 cP, Antioxidant.

**INTRODUCTION**

Herbal extracts have been widely accepted as the potential medicines with less side effects as compared to synthetic drug molecules. In recent years, focus has been directed towards the development of drug delivery system using biologically active compounds derived from the natural sources.1-2 Herbal-based drug delivery systems have long been used in folk medicine, and herbal medicines of natural origin show good therapeutic activity with minimal side effects. The World Health Organization estimates that 80 % of the world’s population currently uses herbal medicines for primary health care. Thus, researchers have begun to focus on herbal drugs and the use of materials of herbal origin. Herbal medicines have many advantages over traditional medicines, including a lower risk of side effects, lower cost, and widespread availability.1-6

Medicinal plants are part of the history of human evolution. More than 50% of all drugs used in modern medicinal treatments are composed of natural products and derivatives thereof. The physicochemical stability is a determining factor in the quality of plant extracts and the transformation of these into dry-powdered form is the most desirable strategy, considering that this form improves its stability and facilitates the manipulation of the material.1-2 Techniques for the incorporation of plant extracts within polymer matrices have indicated a good alternative for the improvement of the functionality of medicinal plant extracts. The spray-drying and solvent evaporation process that involves the dispersion of material inside a coated material is a technique that has been widely used in recent years for the incorporation of extracts into polymer matrices.1-4

*Macaranga* is a genus of the family Euphorbiaceae which comprises of about three hundred species. It is present in some parts of the world which include Indonesia, some parts of Africa, Madagascar, Asia, the east coast of Australia and the Pacific islands.6,7 The *Macaranga gigantea* plants are known to be in the form of shrubs or trees and grow in places with optimum sunlight, secondary forests or forests that have been destroyed. *Macaranga gigantea* plants show several bioactivity which include antitumor, anticancer, antimalaria, antimicrobes,
cyclooxygenase and antioxidant. The plant is also known to have active phytochemicals constituents, especially on its leaves.

Solvent evaporation method has been widely and extensively used to prepare polymeric microparticles containing different drugs and in the development of modified release systems. It is a rapid process that does not involve severe heat treatment; therefore, it is a suitable method to preserve biological products, including temperature sensitive products, without their degradation; it also allows for storage at room temperature. It is an instantaneous process where spherical and uniform samples can be obtained, and the process can be easily scaled up. Thermal degradation, nutrient loss, and denaturation are minimal, allowing maximum retention of the active principles. Solvent evaporation method is a technique more economical than lyophilization (four to seven times less costly); it also allows coating, complex coacervation, and drying of two different feed solutions during the process. The effectiveness of the solvent evaporation method to produce microspheres depends on the successful entrapment of the active agent within the particles, and thus, this process is most successful with drugs which are either insoluble or poorly soluble in the aqueous medium which comprises the continuous phase.

There are different methods to prepare microparticles by solvent evaporation method. The choice of the method that will give rise to an efficient drug encapsulation depends on the hydrophilicity or the hydrophobicity of drug. The O/W emulsion system consists of an organic phase comprised of a volatile solvent with dissolved polymer and the drug to be encapsulated, emulsified in an aqueous phase containing a dissolved surfactant. For insoluble or poorly water-soluble drugs, the oil-in-water (O/W) method is frequently used. Another alternative to prepare microparticles by solvent evaporation method is the water-in-oil-in water (W/O/W) method. The choice of the method that will give rise to an efficient drug encapsulation depends on the hydrophilicity or the hydrophobicity of drug.

Preparation of polymeric microparticles

The solvent evaporation method based on the formation of O/W emulsion was used to prepare microparticles. For the O/W method, ethyl cellulose (Ethocel 10 cp) (10%,15%, 20%) or Eudragit E100 (10%,15%, 20%) were dissolved in dichloromethane. 5 g M. gigantea leaves extract were dissolved in 800 ml aqueous PVA solution (0.25% w/v) containing 0.5 M NaCl and NaOH at pH 12. The emulsion was stirred for 4 h at 500 rpm with a propeller stirrer (Heidolph Elektro GmbH & Co. KG, Kelheim, Germany) to allow microparticle hardening. After 4 h, the microparticles were separated from the external aqueous phase by wet sieving followed by washing with 200 ml deionized water, desiccator-drying for 24 h and storage in a desiccator.

Particle size analysis

Particle size mean and size distribution of the microparticles were measured by Dynamic Light scattering (DLS) (Cilas, 1064 L, France). The appropriate amount of dry microcapsules of each formulation is suspended in deionized water and sonicated for the appropriate time period before measurement. The average diameter of the volume, size distribution and polydispersite of the resulting homogeneous suspension were determined using the DLS technique. The microparticles suspension was dispersed in distilled water and then it was put into the sample chamber of particle size analyzer and measurement of vesicular size was carried out.

Thin layer chromatography (TLC)

Qualitative analysis by thin layer chromatography (TLC) on ethanol extract of M. gigantea leaves, and microcapsule with formula 15% Eudragit E100 polymer (FA3) and formula 15% ethocel 10 cp (FB3) were carried out several times using several eluents with different levels of polarity to obtain a solvent that was able to provide good separation. Spots on the TLC plate were monitored at wavelength 254 nm and 366 nm. Determination of the class of compounds in the TLC test was done by spraying the TLC plate with several reagents using 10% H2SO4 in methanol p.a.
Scanning electron microscopy

The morphology of microparticles was analysed by scanning electron microscopy (SEM). For surface imaging, the microparticles were fixed on a sample holder with double-sided tape. To investigate the inner structure, the particles were spread on transparent tape and then cut with a razor blade. All samples were coated under argon atmosphere with gold to a thickness of 8 nm in a high-vacuum (SCD 040, Bal-Tec GmbH, Witten, Germany). Samples were then analysed on the scanning electron microscope (S-4000, Hitachi High-Technologies Europe GmbH, Krefeld, Germany).

Entrapment efficiency

Microparticles (10 mg) were extracted in 1 ml methanol, followed by agitation in a horizontal shaker (IKA HS 501 digital horizontal Shaker, Janke & Kunkel GmbH & Co. KG IKa Labortechnik, Staufen, Germany) for 2 h (n = 3). 0.1 ml of methanol extract was diluted in 10 ml of pH 7.4 phosphate buffer. The polymer was separated from aqueous solution by filtration using filter paper (Whatman®, GE Healthcare UK Limited, Buckinghamshire, UK). Flavoronoid concentration in the obtained aqueous solution was determined by UV-spectrophotometry at wavelengths of 435 nm (HP 8453 UV-Vis spectrophotometer, Agilent Technologies Deutschland GmbH, Walldbronn, Germany). The actual drug loading and encapsulation efficiency were calculated as follows:

Encapsulation efficiency (%) = (actual drug loading/theoretical drug loading) x 100 %

Antioxidant activity

The free radical scavenging activity of the formulation were measured by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method. Different concentrations of formulations were added to equal volume to methanolic solution of DPPH (100 μM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The mixture was shaken vigorously and allowed to stand at room temperature for 30 minute then the absorbance was measured at 517 nm by using spectrophotometer. Vitamin C was used as standard compound. The percent DPPH scavenging effect was calculated using the following equation:

\[ \text{Inhibition} = \frac{100 \times (A0 - A1)}{A0} \]

Where, A0 was the absorbance of the control reaction and A1 was the absorbance in presence of the standard sample.

RESULT AND DISCUSSION

The phytochemical screening of crude ethanolic extract of M. gigantea leaf revealed the presence of some secondary metabolites such as alkaloids, steroids, flavonoids, phenolics and tannins (Table 1). These phytochemical compounds are known to be responsible for some medicinal activity which in this present study is antioxidant activity.

Microparticle

Preparation of microparticles by solvent evaporation is widely used in pharmaceutical industry. It can be applied for encapsulation of a broad range of substances, from simple drugs to proteins and DNA.23-24 There are several variation of the solvent evaporation technique that have been developed to get efficient drug encapsulation for hydrophilic and hydrophobic drugs. For insoluble or poorly water-soluble drugs, the oil-in-water (O/W) method is frequently used. While for water soluble drugs, the water-in-oil-in-water (W/O/W) method is preferred.24 In case of the preparation of polymeric microparticles for sustained drug release by solvent evaporation technique, the solidification rate is a decisive factor for their release behaviour. A very slow hardening of the emulsion droplets leads to the diffusion of the drug substance out of the droplets and encapsulation efficiency becomes low. Solidification rate of polymeric microparticles during solvent evaporation process influenced solubility of polymers in organic solvents and solubility organic solvent in water, which in turn affects microparticle properties such as particle size, drug incorporation, matrix porosity, solvent residues and initial burst.26-37 Various types of solvent with different physical properties (such as miscibility or solubility, volatility, boiling point, reactivity, viscosity, etc.) have been used to prepare polymeric microparticles. Dichloromethane is the most common solvent for the encapsulation using solvent evaporation technique because of its high volatility, low boiling point and high immiscibility with water.23-24 Microencapsulation techniques with film polymers can use several types of polymers including Eudragit E100 and Ethocel 10 cP. Eudragit E100 is a cationic polymer based on dimethylaminoethyl methacrylate, butyl methacrylate, and methyl methacrylate. Eudragit E100 dissolves in gastric fluid and also in weak acid buffer solutions, up to pH 5. The glass transition temperature of Eudragit E100 is ~ 48°C. If it was used as a polymer cover in microencapsulation, eudragit E100 forms a film that is easily soluble, permeable, and insoluble at pH 5 or higher, but dissolves rapidly by forming salts at acidic pH, lower than 5. This polymer can prevent the release of drugs in saliva at pH 6.8 - 7.4, on the other hand it will dissolve directly in stomach fluid at pH 1.0 - 1.5.28-31 Ethyl cellulose (EC) is a partly O-ethylated cellulose ether derivative. It is available in a variety of grades, which differ in viscosity, usually hydrophobic in nature and widely used in the biomedical and pharmaceutical industries. Ethyl cellulose is usually distinguished by viscosity, molecular weight, and is referred to as "Ethyl Cellulose Polymer Premium", with the trade name Ethocel TM. Ethocel TM types are ethocel 4, 7, 10, 20, 45 and 100 cP. The one used in this research is ethocel 10 cP because it is most used in coating many purposes such as odour and taste masking, protection, and controlled release of drugs.30-31 Ethanol extract of M. gigantea leaves has low stability because it contains natural ingredients. Formulation of extract into microcapsules by using polymer is purported to protect the extract as an active ingredient. The polymer used were eudragit E100 and ethocel 10 cP at concentration of 5, 10 and 15%. PVA in microcapsule prepared by solvent evaporation method is commonly used as a polymer stabilizing agent. PVA has water-soluble properties, slightly soluble in ethanol (95%) besides PVA has a characteristic odor with white granules. However, the use of the polymer must be able to guarantee the stability of the extract, especially in terms of activity.

Based on observations of microcapsules from ethanol extract of M. gigantea leaves using type and concentration of polymer variations, it is obtained that yields of each formulation were 5.024 g for 5% eudragit E100; 5.072 g for 10% eudragit E100; and 5.082 g for eudragit 15% E100. Using Ethocel 10 cP as polymer, the yields values were 5.275 g for 5% ethocel 10 cP; 5.744 g for 10% ethocel 10 cP; and 5.818 g for 15% ethocel 10 cP (Table 2). It can be concluded that the higher the concentration used in formulation, the higher the yield obtained.

Based on the results of the particle size characterization of M. gigantea leaf extract microcapsules, by using 5% Eudragit E100 the particle size was 3.564 μm; when 10% eudragit E100 polymer was used, particle size

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Table 1: Phytochemical screening of ethanolic leaf extract of Macaranga gigantea.

<table>
<thead>
<tr>
<th>Secondary metabolites</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
</tr>
</tbody>
</table>
was 3.998 µm; while 15% Eudragit E100 was used, the particle size was 4.514 µm. The analysis data shows that the particle size is categorized into a micro size (above 1 µm). Formula that used 5% ethocel 10 cP polymer produced a particle size of 3.928 µm. The formula that uses 10% ethocel 10 cP polymer produces a particle size of 4.469 µm. Whereas the formula using 15% ethocel 10 cP produces a particle size of 5.887 µm. From 3 variations of Eudragit E100 and Ethocel 10 cP polymers, it can be seen that at 15% concentration of polymer, largest particle size of microcapsules was the highest compared to that with 5% and 10% (Table 2). But these particle size is still categorized into a micro size (above 1 µm).

Calculation on the % encapsulation efficiency (% EE) aimed to find out how much % of the ethanol extract of M. gigantea leaves can be coated by polymer. In the formula using 5% eudragit E100 polymer, the EE value was 72.746%. By using 10% eudragit E100 polymer, the % EE value was 84.114%, while by using 15% eudragit E100, the % EE value was become 85.978%. The process can be categorized as good because it has the % EE value of ≥ 80%. Microcapsule formula of M. gigantea that use 5% ethocel 10 cP polymer revealed the % EE value as 88.992%. It can be concluded that by using concentration 5-15% of ethocel 10 cP polymer, the value of % EE was categorized as good (Table 2).

Zeta potential

Zeta Potential Analysis is a technique for determining the surface charge of particles in a solution (colloid). Microparticles have a surface charge that attracts a thin layer of charge ions that is opposite to the surface of the microparticles. The double ion layer together with the electrical potential at the bilayer boundary is known as the Zeta potential of the particle and has values that typically range from 100 mV to -100 mV. The magnitude of the zeta potential can predict colloidal stability. Microparticles with a Zeta Potential value greater than +25 mV or less than -25 mV usually have a high degree of stability. Dispersions with low zeta potential value will produce aggregates due to inter-particle Van Der Waals attractions.

Thin layer chromatography

Thin layer chromatography (TLC) is a method of separating components of chemical compounds based on the principle of adsorption and partitioning which is determined by the mobile phase (eluent) and the stationary phase (adsorbent). The use of the TLC method is intended as an initial qualitative analysis of the stability of the active ingredients used. Components of chemical compounds move up to follow the mobile phase because the adsorbent absorption of chemical components is not the same so that chemical components can move at different distances based on the level of polarity. This is what causes the separation of components of chemical compounds in the extract.

The results obtained from the ethanol extract of M. gigantea leaves seen at 366 nm showed the presence of four and five spot with varying Rf values (Figure 2 and Table 3).

<table>
<thead>
<tr>
<th>Formula</th>
<th>Material</th>
<th>Product (g)</th>
<th>PSA (µm)</th>
<th>EE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA₁₁</td>
<td>Extract = 5 g</td>
<td>5.024</td>
<td>3.564</td>
<td>72.746</td>
</tr>
<tr>
<td>FA₁₂</td>
<td>Extract = 5 g</td>
<td>5.072</td>
<td>3.998</td>
<td>84.114</td>
</tr>
<tr>
<td>FA₁₃</td>
<td>Extract = 5 g</td>
<td>5.082</td>
<td>4.514</td>
<td>85.978</td>
</tr>
<tr>
<td>FB₁₁</td>
<td>Ethocel 10 cP = 5%</td>
<td>5.275</td>
<td>3.928</td>
<td>77.995</td>
</tr>
<tr>
<td>FB₁₂</td>
<td>Ethocel 10 cP = 5%</td>
<td>5.744</td>
<td>4.469</td>
<td>86.784</td>
</tr>
<tr>
<td>FB₁₃</td>
<td>Ethocel 10 cP = 15%</td>
<td>5.818</td>
<td>5.887</td>
<td>88.992</td>
</tr>
</tbody>
</table>

**Annotation:**
- FA₁₁ = Formula with eudragit E100 (5%)
- FA₁₂ = Formula with eudragit E100 (10%)
- FA₁₃ = Formula with eudragit E100 (15%)
- FA₂₁ = Formula with ethocel 10 cP (5%)
- FA₂₂ = Formula with ethocel 10 cP (10%)
- FA₂₃ = Formula with ethocel 10 cP (15%)
- PVA = Polivinyl Alkohol
- PSA = Particle Size Analyzer
- EE = Encapsulation efficiency
Figure 1: Zeta potential profile of *M. gigantea* leaves ethanol extract microcapsule, (a) FA3 = Eudragit E100 Formula (15%); (b) FB3 = Ethocel 10 cP Formula (15%).

Table 3: Rf value in thin layer chromatography profiles.

<table>
<thead>
<tr>
<th>Rf Value</th>
<th>Extract</th>
<th>FA3</th>
<th>FB3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.39</td>
<td>0.6</td>
<td>0.49</td>
</tr>
<tr>
<td>2</td>
<td>0.49</td>
<td>0.5</td>
<td>0.31</td>
</tr>
<tr>
<td>3</td>
<td>0.34</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>4</td>
<td>0.26</td>
<td>0.27</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>0.15</td>
<td>0.18</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Figure 2: TLC profile of *M. gigantea* leaves ethanol extract; FA3 = Eudragit E100 Formula (15%); FB3 = Ethocel Formula 10 cP (15%) under UV light 366 nm.
It can be seen from the results of TLC (Figure 2), the presence of polymers as excipient in microencapsulation process did not affect the stability of compounds in the ethanol extract of *M. gigantea* leaves. So it can be concluded that there were no incompatibility between the extract and polymer (E100 Eudragit polymer (FA3) and ethocel 10 cP (FB3)) after they were formulated into microcapsules.

**Scanning electron microscope**

Microcapsules refer to particles with a diameter of 1-1000 µm. Microcapsules are usually described as a mixture consist of active substances and polymers. Based on the results of particle size and % EE evaluations, formula using 15% eudragit E100 polymer (FA3) and the formula using 15% ethocel 10 cP polymer (FB3) were assumed as best formulation. Therefore, the two formula were subjected to next characterization which was SEM picture analysis.

Results of Scanning electron microscopy revealed that microcapsules using Eudragit E100 polymers have a rough and porous surface (Figure 3. a1 and a2) while ethocel 10 cP has a smoother surface and less visible pores (Figure 3. b1 and b2). The microcapsules using eudragit E100 polymer have a particle size of 4.514 µm while those using ethocel 10 cP polymer have a particle size of 5.882 µm. The resulting particle size has met the size requirements of the microcapsules.

**Antioxidant activity**

The antioxidant reacts with stable free radical, DPPH and converts it to 1, 1-Diphenyl-2- Picryl Hydrazine. The ability to scavenge the free radical, DPPH was measured at an absorbance of 517 nm. So the DPPH –RSA and its %inhibition of ethanol extracts of *M. gigantea* leaves that IC₅₀ values 63.75 ppm and microcapsule showed that IC₅₀ values 64.51 ppm (Table 4). Ascorbic acid (Vitamin C) has taken as reference which showed 7.24 ppm (Table 4). The Results showed that both ethanolic extracts of *M. gigantea* and microcapsule containing extracts of *M. gigantea* leaves have potent antioxidant activity. According to Molyneux (2004), parameters for antioxidant testing based on the ability to inhibit free radicals 50% are categorized if < 50 (Very Strong), 50-100 (Strong), 101-150 (Medium), > 150 (Weak). The ability of vitamin C can inhibit free radical activity and is classified as very strong (IC₅₀ < 50 ppm), due to more hydroxyl groups in its structure, so it can stabilize free radicals. Antioxidant ability of ethanol extracts of *M. gigantea* leaves expressed as IC₅₀ values was 63.75 ppm, while that after formulated into microcapsule showas 64.51 ppm. The results showed that microencapsulation process did not affect the antioxidant activity of the extract. This activity may due to many compounds either as extract or microcapsule that are possible as a source of free radical stabilizing hydroxyl groups. Antioxidant activity is influenced by the number of hydroxyl groups that are able to donate hydrogen atoms to neutralize free radicals. The results was supported by TLC profile of extract and microcapsules which remained stable and revealed the same spots. This means that formulation process and addition of the coating polymer do not affect the pharmacological stability of extract.

The antioxidant activity of the extract is due to secondary metabolite content. In phytochemical screening, extracts have secondary metabolites, namely alkaloids, flavonoids, tannins, phenolic and steroids. These compounds are responsible for the antioxidant activity.
CONCLUSION

Based on the results of the data, it can be concluded that microcapsules of M. gigantea leaves extract can be prepared by solvent evaporation method (O/W: oil in water) by using Eudragit E100 and Ethocel 10 cP as polymer. Characterization of the microcapsules revealed that parameter process used on this method is applicable to produce microcapsules which stable in physical properties and also in pharmacological activity as antioxidant.

ACKNOWLEDGEMENT

The authors gratitude the Directorate General of Higher Education; the Ministry of Education and Culture and the Ministry of Research and Technology, Republic of Indonesia for their support on this project under the Excellent Research of University Grant year 2020.

CONFLICTS OF INTEREST

No conflicts of interest is associated with this work.

REFERENCES

GRAPHICAL ABSTRACT

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