International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Issue 6, 2023

Original Article

MICROENCAPSULATION OF *SCHLEICHERA OLEOSA* L. LEAF EXTRACT IN MAINTAINING THEIR BIOLOGICAL ACTIVITY: ANTIOXIDANT AND HEPATOPROTECTIVE

ZULHAM[1,4], YOGA WINDHU WARDHANA[1], ANAS SUBARNAS[2], YASMIWAR SUSILAWATI[3], ANIS YOHANA CHAERUNISAA[1*]

[1]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Padjadjaran University, Indonesia. [2]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Padjadjaran University, Indonesia. [3]Department of Pharmaceutical Biology, Faculty of Pharmacy, Padjadjaran University, Indonesia. [4]Sekolah Tinggi Ilmu Farmasi Makassar, Makassar 90242, Indonesia
[*]Corresponding author: Anis Yohana Chaerunisaa; [*]Email: anis.yohana.chaerunisaa@unpad.ac.id

Received: 24 Jul 2023, Revised and Accepted: 26 Aug 2023

ABSTRACT

Objective: *Schleichera oleosa* L. leaf extract has been studied to have antioxidant activity due to the presence of phenolic compounds, especially flavonoids. Flavonoid compounds that have potential as antioxidants are generally unstable and rapidly degraded due to the influence of moisture, heat, light, oxygen, and other reactive components. Microencapsulation is an effective method for maintaining the stability of bioactive compounds. This study aims to formulate *S. oleosa* leaf extract microcapsules and test their stability based on the results of physical characterization, antioxidant, and hepatoprotective activities.

Methods: The microencapsulation process of *S. oleosa* leaf extract was carried out using a fluid bed coating using a polyvinyl alcohol matrix and Ethocel 10 cP. Stability test using a climatic chamber at 40 °C for 90 d. Physical characteristics consist of drying shrinkage, flow rate, angle of repose, compressibility, particle size, and scanning electron microscope (SEM) picture. Antioxidant activity was tested *in vitro* using the *2,2-diphenyl-1-picrylhydrazyl* (DPPH) method, and hepatoprotective activity was tested using the paracetamol-induced hepatotoxicity method in rats.

Results: The results showed that the microcapsules, after stability testing, could maintain the total phenolic content and antioxidant activity in the strong category with IC_{50} values ranging from 50 to 100 ppm. The results of the hepatoprotective activity test of *S. oleosa* leaf extract microcapsules tested on days 0 and 90 (after stability testing) could significantly reduce SGOT and SGPT levels compared to negative controls after being induced with paracetamol. Still, this effect was lower than that of curcumin, which is known to be hepatoprotective.

Conclusion: The application of the microencapsulation method to *S. oleosa* leaf extract plays an essential role in maintaining physical stability and maintaining its biological activity as an antioxidant and hepatoprotector.

Keywords: Microencapsulation, *Schleichera oleosa* L., Polyvinyl alcohol, Ethocel 10 cP, Fluid bed coating, Antioxidant, Hepatoprotective

© 2023 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (https://creativecommons.org/licenses/by/4.0/)
DOI: https://dx.doi.org/10.22159/ijap.2023v15i6.48960. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

In Indonesia, *Schleichera oleosa* L. is a type of plant that is often used in traditional medicine. Several studies have shown that *S. oleosa* leaves can be used as a medicinal plant. *S. oleosa* leaf extract has been studied and has antioxidant activity due to the presence of phenolic compounds, especially flavonoids. A phytochemical analysis of *S. oleosa* leaves showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, saponins, and steroids. This was one of the research results on the activity of the *S. oleosa* plant. Phenolics and flavonoids are the most abundant bioactive compounds in *S. oleosa* leaves [1]. The results of this study indicate that the leaves contain many bioactive compounds, and the presence of these phytochemicals, mainly phenols and flavonoids, is the reason for the use of *S. oleosa* in ethnomedicine to treat various diseases. Secondary metabolites in *S. oleosa* leaves are flavonoids, alkaloids, tannins, phenols, and steroids [1]. The results of the study showed the antioxidant activity of the *S. oleosa* leaf extract fraction with IC_{50} values, namely the ethyl acetate fraction of 206.0851 ppm, the water fraction of 272.2891 ppm, and the n-hexane fraction of 425.1143 ppm, which means that the ethyl acetate fraction has an inhibitory effect and produces more potent radicals than the water and n-hexane fractions [2].

Reactive Oxygen Species (ROS) are free radicals that affect various biological and pathophysiological processes, including liver disease, diabetes mellitus, heart disease, cancer, kidney failure, brain dysfunction, neurodegenerative diseases, decreased immune systems, inflammation, stress, and aging [3, 4]. Oxidative stress can cause lipid peroxidation, leading to cell damage and degenerative diseases such as liver disease [5]. Protection by antioxidants can avoid these diseases through a complex protection system that works synergistically to neutralize free radicals. Hence, the free radicals will be stabilized or deactivated before they attack the cells [6]. Antioxidants are one of the hepatoprotective mechanisms; damage to cell membranes and proteins is a sign of oxidative stress caused by free radicals [4].

One type of antioxidant based on its mechanism is phenolic compounds and their derivates, which cover a large phytochemical group in plants, including flavonoids [7]. Flavonoids have been shown to have antioxidant activity, free radical scavenging capacity, and help prevent oxidative stress, coronary heart disease prevention, hepatoprotective, anti-inflammatory, anticancer, and antiviral activity [8].

The relevant antioxidant activity of bioactive substances can be inhibited due to degradation by light, oxygen, temperature, humidity, and unsaturated bonds in the molecular structure. Natural antioxidant compounds are generally unstable, and the application of these bioactive compounds to new products is still minimal. The application of microencapsulated bioactive compounds as functional applications shows significant potential where most of the core material is covered by coating material, thereby providing several advantages and covering the disadvantages of the core material [9]. The characteristics of antioxidants allow their use in food, cosmetics, and pharmaceuticals to increase the shelf life of these products, reduce the use of synthetic antioxidants, and promote their beneficial effects on the human body [10].

Encapsulation is a technique in which a material or mixture is coated with another system or material; therefore, a suitable coating material is required to produce microparticles. The type of polymer used will determine the characteristics of the resulting microparticles because the coating or encapsulation system is designed to protect the active agent from the adverse effects of moisture, heat, light, oxygen, and other reactive components present

in the matrix [11, 12]. It is, therefore, essential to choose a coating material that can maintain stability during the conditions of manufacture and storage [9, 13].

Microencapsulation provides several advantages, including protecting active substances against environmental influences, masking bad taste, maintaining volatiles or cell viability, separating incompatible substances, protecting the body from side effects, and optimizing, extending, or targeting drug effects by trapping them in a coating material. Protective coating forms particles with a particle diameter of several nanometers to several millimetres [13-15]. Thus, microencapsulation with appropriate carriers is an alternative technology to increase bioactive storage and environmental stability, providing another advantage and masking flavours.

Various methods are used in the microencapsulation process, namely chemical and mechanical processes. The mechanical methods used during microencapsulation include spray drying, spray chilling, fluidized bed coating, spinning discs, and pan coating [16]. One example of an encapsulation technique is fluidized bed coating, which is based on a drying process due to the liquid nature of extracts containing bioactive compounds [17].

The novelty of this research is the effort to protect the active substance of the extract from environmental influences to protect its antioxidant activity. This is because the antioxidant activity is thought to be due to the presence of phenolic compounds which are known to be unstable to light and oxidation, so they need to be protected physically to maintain their activity. An important method for maintaining the stability of bioactive compounds by using encapsulation technology, which helps protect compounds from adverse conditions during the process of storage [18]. This study examines the process of making *S. oleosa* extract microcapsules using a fluid bed coating tool using a variety of hydrophilic and hydrophobic polymers. The hydrophilic polymer used was polyvinyl alcohol, while the hydrophobic polymer used was ethyl cellulose (Ethocel 10 cP). The resulting microcapsules were tested for physical stability, antioxidant activity, and hepatoprotective effect, which were tested on days 0 and 90 (after storage in the climatic chamber).

MATERIALS AND METHODS

Plant samples and extract preparation

Schleichera oleosa L. was obtained from Takalar Regency, South Sulawesi Province. The plant of *S. oleosa* was determined at the Indonesian Institute of Sciences, Centre for Biological Research, Botany-LIPI Bogor, Indonesia (No: 726/IPH.1.01/If.07/VII/2020). Samples were washed, chopped, and dried in a simplicia drying cupboard, sorted dry, and then powdered until *S. oleosa* leaf simplicia was obtained.

The simplicia was extracted using 70% ethanol solvent using the maceration method [19]. The simplicia is put into the maceration container then enough solvent is added for the wetting process. The remaining solvent was added until all the Simplicia was submerged entirely and left in a place protected from sunlight for three days while stirring occasionally. The filtrate is filtered, and the residue is macerated again with the same solvent. Maserati was concentrated using a rotary evaporator at 70 °C with a rotational speed of 75 rpm until a liquid extract was obtained.

Animals

Animals used in hepatoprotective activity were 2-3 mo healthy male white rats strain *Wistar* (n = 30), weighing 220-260 grams. During the quarantine period, the body weight should increase or remained constant. The animals were obtained from the School of Life Science and Technology, Bandung Institute of Technology, Indonesia. Before treatment, the animals were adapted for one week provided by adequate food and drink under a 12 h light/dark cycle at room temperature. All experimental procedures were approved with the Padjadjaran University Research Ethics Committee (498/UN6. KEP/EC/2020).

Research procedure

Microencapsulation

The process of making microcapsules using a fluid bed coating tool The first stage was the granulation process of the ethanol extract of *S. oleosa* left using lactose as the core and coated with hydroxypropyl methylcellulose (HPMC) with a concentration of 10% as a binder. The next step is the granule coating process using polyvinyl alcohol polymer and Ethocel 10 cP, each with a concentration of 10%. Process parameters for coating the equipment, such as outlet temperature (37.2 °C), spray interval (10 s), product temperature (37,8 °C), inlet temperature (37,8 °C), and spray speed (20.5 rpm) [20].

Microcapsules physical characterization

The physical characterization of microparticles includes shrink drying, flow rate, rest angle, and compressibility. The particle size of the microcapsules was analyzed using the Beckman Coulter LS 13 320 Particle Size Analyzer, and a scanning electron microscope (SEM) observed surface shape and morphology.

Determination of total phenolic content

The total phenolic content was determined using a spectrophotometric method [21]. First, 10 g of microcapsules were dissolved in ethanol p. a.; the volume was made up to 25 ml and centrifuged at 6000 rpm for 15 min. Then pipetted 500 µL of the filtrate and added 1.25 ml of 10% Folin-Ciocalteau reagent and 1 ml of 7.5% Na_2CO_3. The solution was allowed to stand for 15 min in a water bath at 45 °C; then, the absorbance was measured at a maximum wavelength of 756 nm. Repeat the gallic acid standard measurement and construct a standard curve to determine the concentration of the sample. The standard curve for gallic acid has concentrations of 20, 40, 60, 80, and 100 ppm.

Antioxidant activity test

Antioxidant activity was determined based on free radical inhibition of the ethanol extract of *S. oleosa* leaves using the *2,2-diphenyl-1-picrylhydrazyl* (DPPH) method [22]. Extract samples were prepared by a series of dilutions in ethanol (5, 10, 15, 20, and 25 ppm). Then 1.5 ml of a 160 ppm DPPH solution was added to each extracted sample up to 1.5 ml. The mixture was shaken vigorously and incubated at room temperature for 30 min. The absorbance of the mixture was then measured at 516 nm using a UV-VIS spectrophotometer in triplicate. The control group only contained 2 ml of 160 ppm DPPH and 2 ml of ethanol PA without extract. Ascorbic acid was used as the reference standard compound. Radical scavenging activity or percent inhibition is calculated as follows:

$$\% \text{ inhibition} = \frac{\text{Control absorbance} - \text{Sample absorption}}{\text{Control absorbance}} \text{ x } 100\ \%$$

After obtaining the percent inhibition of each concentration, y = A+Bx is determined by calculating linear regression, where x is the concentration (g/ml), and y is the percentage inhibition (%). Thus, antioxidant activity is expressed by the Inhibition Concentration of 50% (IC_{50}), which is the sample concentration that can reduce DPPH radicals by 50%. The IC_{50} value is obtained from the x value after replacing y with 50.

Hepatoprotective activity

The rat were quarantined and acclimatized for for six days, and at the end of the adaptation period, serum analysis was carried out in all groups to determine the normal state of SGPT and SGOT levels. For seven consecutive days, the healthy control (NC) and the negative control (NEC) group received orally 2% gum arabic suspension, while the positive control (PC) was given curcumin 100 mg/kg body weight. In the same time, the treatment groups (MIC-1, MIC-2 and MIC-3) were given microcapsule orally with doses 100 mg/kg body weight. On the eighth and ninth days, the NC, PC and the extract dose groups were given paracetamol orally at a dose of 1000 mg/kgBW. The healthy control group (HC), was given only food and water. On day 10^{th}, all animals were anesthetized, and blood was drawn through the orbital sinus of the eye. Blood samples were stored in blood tubes, and then the serum was separated by centrifugation at 3000 revolutions/minute for 15 min. Serum samples were used for the final measurement of SGOT and SGPT levels by adding bioassay marker enzymes, glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT), and then measuring the absorbance using a UV spectrophotometer at a wavelength of 340 nm.

Statistical analysis

The difference in the average levels of SGOT and SGPT between treatment groups was statistically analyzed using SPSS 24, and statistical significance was determined by one-way analysis of variance (ANOVA) and post hoc least significant difference (LSD) tests, with a P-value of less than 0.05 considered significant. An analysis was carried out on the results of measuring SGOT and SGPT levels on days 0 and 90. A comparison of SGOT and SGPT levels on days 0 and 90 used a paired t-test to find out the average difference before and after being given treatment.

RESULTS AND DISCUSSION

Microencapsulation

The *S. oleosa* extract was subjected to a microencapsulation process employing fluid bed coating, a technique for mechanically creating microcapsules using two coating types: polyvinyl alcohol and ethyl cellulose (Ethocel 10 cP).

Microencapsulation is the process of packaging a solid, liquid, or gas as an active ingredient with a continuous film layer as a coating to form a capsule, where the functional material is protected from its environment by adsorbing it in a protective coating material and forming particles with diameters ranging from millimeters to nanometers [13, 15].

In the pharmaceutical industry, microencapsulation has a lot of benefits for the controlled release of bioactive compounds and therapeutic enzymes taken by mouth. This protects by covering these sensitive compounds with different coating materials, facilitating long-term stability and preventing the loss of active ingredients. Several results have shown that the microencapsulation process is technically and economically feasible for therapeutic applications for many bioactive compounds and plant extracts. The advantages of microencapsulation include ease of distribution and handling, the possibility of being compressed in packaging to save space, and protection against oxidation at room temperature. Encapsulation can change the liquid form to a solid form that is stable and free-flowing, making it easy to handle and apply in the formulation process [14, 23].

Microcapsules can be formulated using various techniques. However, many microencapsulation techniques share common characteristics. Microencapsulation techniques can be classified as chemical processes such as the interfacial method and in-situ polymerization, physiochemical processes such as coacervation-phase separation and complex emulsions, electrostatic processes, mechanical processes such as the air-suspension method, pan coating and spray drying, spray congealing, micro-orifice systems, and the rotary fluidization bed granulator method [24].

Fluidized bed coating is also known as suspension coating. This technique is mainly used in the pharmaceutical and food industries due to its benefits. The principle involved in fluidized bed coatings is that the liquid coating or wall material is sprayed onto the core particles, and the rapid evaporation helps the formation of a protective layer on the active material or core [17].

A fluidized bed coating a dryer that uses the principle of fluidization. The working principle of the FBD system dryer is the blowing of controlled hot air with a particular volume and pressure by a blower through a channel into the drying tub. The airflow causes the particles to rise and rotate within the bed. At the same time, under the pressure of the spray, the polymer solution is atomized. It sticks to the particles so that it can continuously coat the surface of the particles, and a drying process occurs to form a polymer layer. In this process, drug particles are coated and dried while suspended in an upward-moving air current. Solutions and suspensions of coating materials in water and volatile organic solvents are used. Drying of the coated particles is carried out at room temperature or an elevated temperature, depending on the solvent used [25, 26].

The coating or encapsulation process is designed to protect the core material from environmental factors that may cause deterioration and to prevent premature interaction between the core material and other food components, which is very important when selecting a coating material that is stable during processing and storage conditions [13].

Microcapsules were obtained from 2 different types of coatings, namely polyvinyl alcohol with a coating efficiency of 87.79% and ethocel 10 cP with a coating efficiency of 84.88%, to determine the effect of the microencapsulation process on the stability of the content of bioactive compounds contained in the *S. oleosa* extract, an evaluation was carried out, including the total phenol content, antioxidant activity, and physical characteristics of the microcapsule, which had been obtained from 2 types of coatings, namely polyvinyl alcohol and ethyl cellulose (Ethocel 10 cP).

Microcapsules physical characteristics

Examination of the physical characteristics of microcapsules consists of flow rate, angle of repose, compressibility, particle size, and morphology.

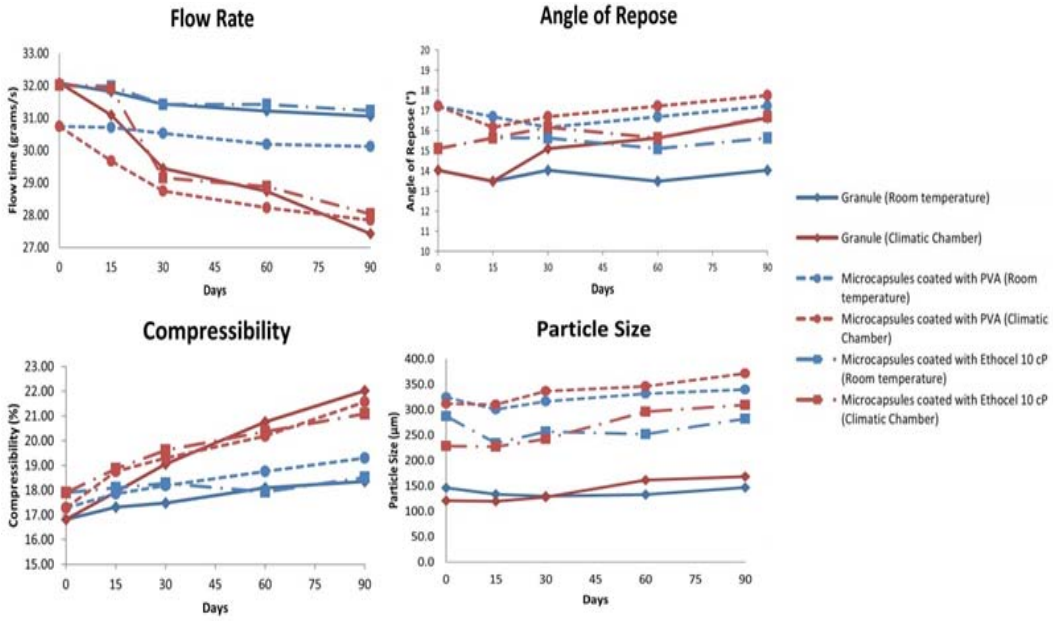


Fig. 1: Effect of temperature conditions on the physical characteristics of *s. oleosa* microcapsules

The flow rate of granules, microcapsules coated with polyvinyl alcohol, and Ethocel 10 cP under storage conditions in a climate chamber decreased. This affected the increase in the value of the angle of repose of granules and microcapsules, which were coated with polyvinyl alcohol and Ethocel 10 cP (fig. 3).

The compressibility of granules, microcapsules coated with polyvinyl alcohol, and 1 Ethocel 10 cP when stored in a climate chamber showed an increase in compressibility values compared to storage conditions at room temperature (fig. 3).

Based on the measurement results, the particle size of granules and microcapsules coated with polyvinyl alcohol and Ethocel 10 cP increased compared to storage conditions at room temperature but still met the requirements in the microparticle size range (fig. 3). Microcapsules, generally 1–1000 μm, serve as multiunit drug delivery systems with well-defined physiologic and pharmacokinetic benefits to enhance efficacy, tolerability, and patient adherence.

The morphological shape of microcapsules coated with Ethocel 10 cP shows a flatter surface (fig. 5) compared to microcapsules coated with polyvinyl alcohol, which offer an uneven surface (fig. 4) observed before and after testing during the period up to the 90th day.

The physicochemical properties of the made microcapsules depend mainly on the material used to make them. The encapsulating material must be stable, non-hygroscopic, and capable of producing a cohesive film with the core material. This should provide the desired coating properties, such as strength, brittleness, flexibility, impermeability, and stability. Some encapsulation materials or matrices may be selected depending on the desired properties of the final microcapsule, including stability and unchanged bioavailability, compatibility with the chosen microencapsulation technique, and high encapsulation efficiency. Good film-forming properties, low viscosity when present in solution or suspension, and desirable gelling and barrier properties [24, 27].

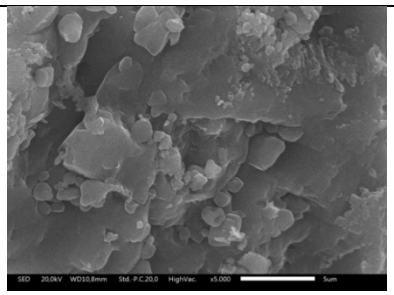
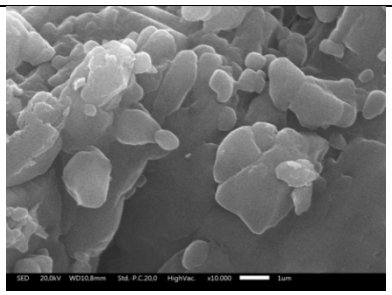
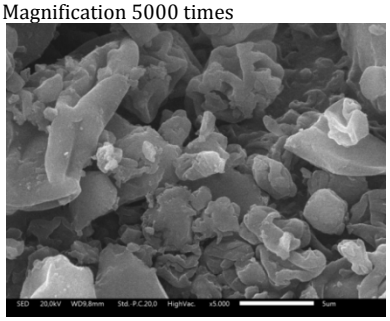
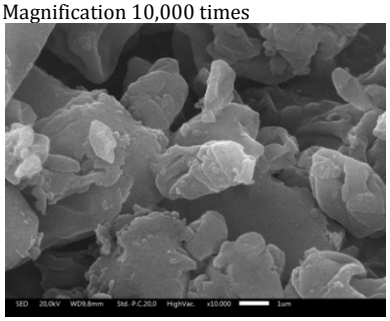
Microcapsules (Polyvinyl alcohol polymer) Day 0	 Magnification 5000 times	 Magnification 10,000 times
Microcapsules (Polyvinyl alcohol polymer) 90th day	 Magnification 5000 times	 Magnification 10,000 times

Fig. 2: Results of SEM analysis of *S. oleosa* microcapsules coated with polyvinyl alcohol

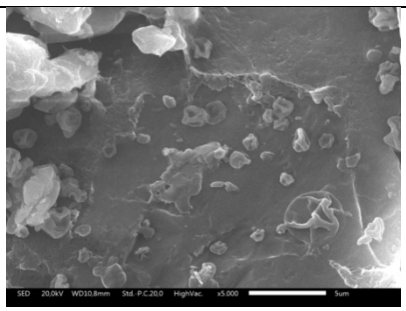
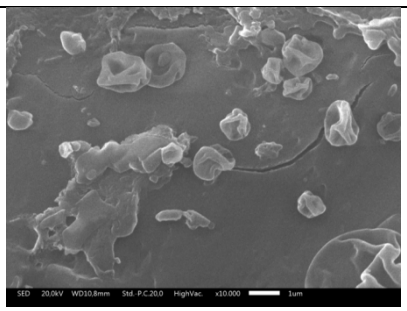
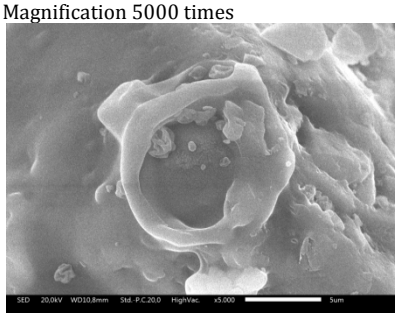
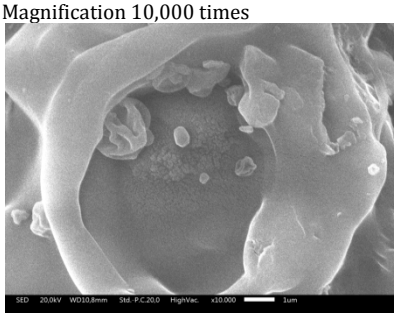
Microcapsule (Polymer ethocel 10 cP) Day 0	 Magnification 5000 times	 Magnification 10,000 times
Microcapsule (Polymer ethocel 10 cP) 90th day	 Magnification 5000 times	 Magnification 10,000 times

Fig. 3: SEM analysis results of *S. Oleosa* microcapsules coated with Ethocel 10 cP

The critical factor in the microencapsulation process with a fluid bed coating device is that the coating concentration should be manageable because it will cause the granules to become sticky so that they will form agglomerates, which affect the shape and morphology of the resulting granules. In addition, the setting of the tool is essential, especially the determination of the inlet temperature, to produce a perfect granule drying process. The drying technique and the material used as the coating usually affect the retention capacity of the compound in the matrix. Therefore, it is crucial to correctly choose the coating materials and encapsulation techniques to maximize the incorporation and retention of functional compounds in the encapsulation matrix [17].

Microcapsule total phenol content

The total phenolic content of microcapsules coated with polyvinyl alcohol polymer and Ethocel 10 cP, both at room temperature and in a climatic chamber, showed relatively the same levels under storage conditions. Different things were shown for granules that were not well coated at room temperature storage conditions, and storage in the climatic chamber experienced a decrease in the amount of total phenol content during storage conditions because the granules did not go through the encapsulation process, so there was no protection for the bioactive compounds, so the decomposition of substances, both secondary metabolites and other bioactive compounds, occurred. These results indicate the effect of encapsulation technology on granules to maintain the phenol content during the process until storage conditions (fig. 4).

Microcapsule antioxidant activity

The antioxidant activity of microcapsules coated with polyvinyl alcohol and Ethocel 10 cP, both under storage conditions at room temperature and in a climatic chamber, showed relatively stable activity during the process and under storage conditions. In contrast, uncoated granules, both at room temperature and in climatic chamber storage conditions, showed a decrease in antioxidant activity during storage conditions, which correlated with a decrease in total phenolic content, which is a bioactive compound as an antioxidant. The decrease in antioxidant activity was seen in the increasing IC_{50} value of uncoated granules (fig. 4).

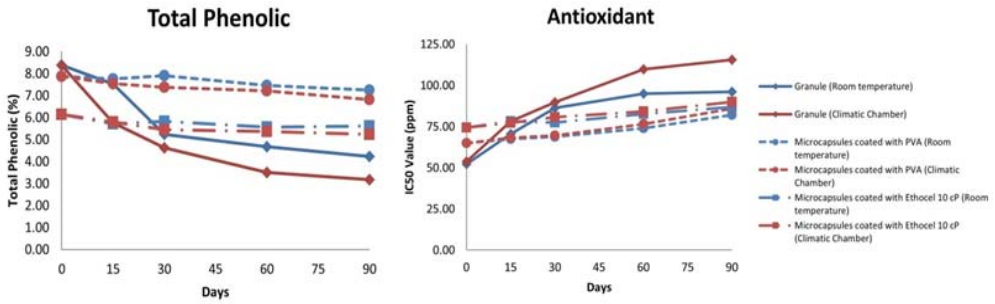


Fig. 4: Effect of temperature conditions on total phenolic content and antioxidant activity of *S. oleosa* microcapsules

Based on the results of measurements of total phenolic content and activity in microcapsules carried out at 0, 30, 60, and 90 d, microcapsules with polyvinyl alcohol coatings, although they have greater coating efficiency than ethocel 10 cP coatings, show a percent decrease in the concentration of phenol levels. The total and IC_{50} values were greater than those of the microcapsules coated with 10 cP ethocel. This could be due to differences in the characteristics of the materials and their interactions with the extracts up to the microencapsulation stage.

Some examples of the types of coating materials, such as proteins, sugars, starches, gums, lipids, and cellulose derivatives, are the most frequently used [13]. Various types of natural and synthetic polymers, which can form semipermeable membranes under certain conditions, are used to make microcapsule shells, and one of them is polyvinyl alcohol (PVA) [28]. Polyvinyl alcohol (PVA), perhaps one of the most promising materials, is a highly polar, non-toxic, water-soluble synthetic polymer prepared by the hydrolysis of polyvinyl acetate and used in polymer blends with natural polymeric materials. In addition, it is used for various biomedical applications due to its non-toxicity, non-carcinogenicity, good biocompatibility, desirable physical properties, and excellent film-forming properties [29].

Ethyl cellulose is a derivative of cellulose in which some of the hydroxyl groups in the anhydroglucose units are repeatedly modified into ethyl ether groups, mainly referred to as the non-ionic ethyl ether of cellulose. Ethylcellulose has been widely used for microencapsulation because it has many versatile biocompatible properties: it is compatible with many celluloses, resins, and almost all plasticizers; it is non-biodegradable, so it is only used in oral formulations; it is stable to light, heat, oxygen, moisture, and chemicals; it is non-toxic and non-irritating; its thin film exhibits good flexibility and mechanical strength over a wide range of temperatures; it cannot expand; and it is insoluble in water. So the compactness and porosity of ethyl cellulose play a crucial role in drug release from the hydrophobic material. Although ethyl cellulose is insoluble in water, it can absorb water [24]. Ethyl cellulose is available in various grades that differ in viscosity, are usually hydrophobic, and are widely used in the biomedical and pharmaceutical industries. Ethyl cellulose is usually distinguished by its consistency and molecular weight and is referred to as "Ethyl Cellulose Polymer Premium" under the trade name Ethocel TM. The types of Ethocel TM are Ethocel 4, 7, 10, 20, 45, and 100 cP [27, 30]. The type of ethyl cellulose used in this study was Ethocel 10 cP because it is most widely used in coatings for various purposes, such as odour and taste masking, protection, and controlled drug release.

Table 1: Results of measurement of SGOT and SGPT levels in rats on Days 0 and 90 (After being stored in the climate chamber)

No.	Treatment	Day 0		Day 90	
		SGOT	SGPT	SGOT	SGPT
1.	NC	83.69±5.42	46.99±2.99	80.86±3.27	46.67±4.21
2.	NEC	216.07±8.89	119.59±12.05	216.76±3.81	113.42±8.48
3.	PC	95.25±3.05	65.96±5.88	94.02±8.73	58.22±0.92
4.	MIC-1	117.41±6.11	80.06±4.36	184.41±5.30	93.89±2.69
5.	MIC-2	113.18±7.01	78.72±4.46	109.35±4.25	75.78±1.95
6.	MIC-3	109.11±2.82	74.76±6.43	109.14±2.60	79.43±1.87

NC: Normal control; NEC: Negative control; PC: Positive control; MIC-1: Uncoated granules; MIC-2: Microcapsule coated with Polyvinyl alcohol; MIC-3: Microcapsule coated with Ethocel 10 cP.

Hepatoprotective activity

After being given paracetamol, the negative control group (NEC) experienced a significant increase in SGOT and SGPT levels (table 1). These results indicate that paracetamol causes liver damage, which is characterized by increased levels of SGPT and SGOT. Thus, the positive control group (PC) revealed lower SGOT and SGPT than the negative group. Thus, curcumin as a positive control, was shown to have a protective effect on the liver and was mainly related to its antioxidant properties [31].

The hepatoprotective activity of *S. oleosa* leaf extract was evaluated using the paracetamol-induced hepatotoxicity method because paracetamol has long been known to cause acute liver toxicity. At therapeutic levels, paracetamol is metabolized to a highly reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), by cytochrome P450 enzymes in liver cells. This metabolite is associated with the intracellular antioxidant glutathione (GSH) to form a non-toxic metabolite, which is excreted by the kidneys. However, at high doses of paracetamol, NAPQI is produced in large quantities sufficient to deplete GSH and cause hepatic necrosis [32, 33]. Injured hepatocytes release the enzymes SGOT and SGPT into the blood vessels. The activity of these two enzymes can be measured to indicate liver dysfunction [34, 35]. Higher levels of SGOT and SGPT indicate greater liver damage. In this study, paracetamol at a dose of 1000 mg/kg increased SGOT levels significantly compared to normal controls; this indicates that paracetamol induces toxicity in liver cells.

The ANOVA test followed by the LSD (Least Significant Difference) test showed that the negative control group had a significant difference between all groups (fig. 5). The sample group that was given the treatment (MIC-1, MIC-2, and MIC-3) showed significantly different levels of SGOT and SGPT statistically than the negative control group, which experienced significant increases in levels of SGOT and SGPT. This shows that the administration of microcapsule preparations can repair liver cells damaged by paracetamol induction in animals. The hepatoprotective activity shown by microcapsules may be due to their antioxidant activity.

Then, the hepatoprotective activity was tested for the microcapsules on the 0th day and the 90th day for the microcapsules (which had been stored in a climatic chamber at a temperature of 40 °C±2 °C with a relative humidity of 75%±5%). The results of measuring SGOT and SGPT levels were then followed by correlation testing. The correlation results showed that between the microcapsule group on day 0 and the microcapsule group on day 90, the correlation was marked with a significant value greater than the value α = 0.05.

The results of the paired-sample T-test data between the microcapsule group coated with polyvinyl alcohol on day 0 and the microcapsule group coated with polyvinyl alcohol on day 90 showed a significance value greater than the value α = 0.05, which means that there was no difference in the average value of SGOT and SGPT between the microcapsule group on day 0 and the microcapsule group on day 90, both microcapsule coated with polyvinyl alcohol and microcapsule coated with Ethocel 10 cP.

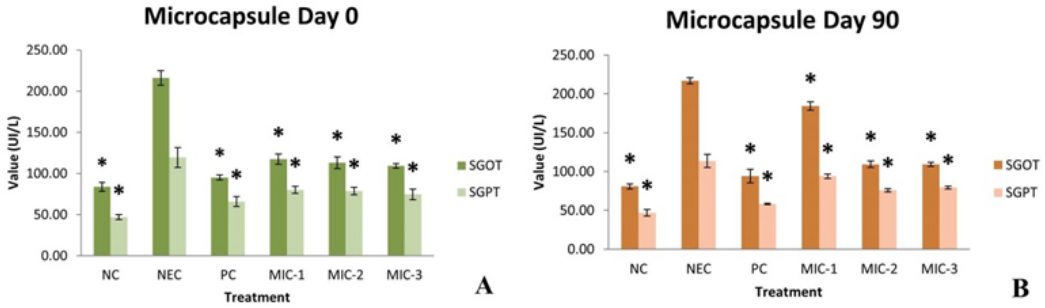


Fig. 5: Effect of the microcapsule *S. oleosa* leaf extract on SGOT and SGPT levels of the rat. A: Microcapsule day 0, B: Microcapsule day 90 (after being stored in the climatic chamber). *p<0.05 significant when compared to the negative control group. NC: Normal control; NEC: Negative control; PC: Positive control; MIC-1: Uncoated granules; MIC-2: Microcapsule coated with polyvinyl alcohol; MIC-3: Microcapsule coated with Ethocel 10 cP

In contrast to the uncoated granules, the correlation results showed that the uncoated granules group on day 0 and the uncoated granules group on day 90 correlated with each other with a significant value greater than the value α = 0.05. The results of the paired-sample T-test data between the uncoated granules group on day 0 and the uncoated granules group on day 90 showed a significance value lower than the value α = 0.05, which means that there was a difference in the average values of SGOT and SGPT levels between the uncoated granules group on day 0 and the uncoated granules on day 90, which still showed increased levels of SGOT and SGPT. This can be caused because the granules are not coated, so there is no protection against the bioactive compounds. There is a storage treatment in the Climatic chamber for 90 d to evaluate its stability, where there is an increase in temperature and humidity that can accelerate the decomposition of substances, both secondary metabolites and other bioactive compounds.

The application of microencapsulation technology to *S. oleosa* extract showed its effectiveness in maintaining total phenolic content and antioxidant activity (IC_{50} value) compared to uncoated extracts. Microcapsules using polyvinyl alcohol polymer and Ethocel 10 cP showed relatively stable chemical stability regarding total phenol content and antioxidant activity. However, it shows differences from the results of physical stability testing, namely flow rate, angle of repose, particle size, and compressibility. Microcapsules coated with Ethocel 10 cP also had a more uniform shape and smoother surface morphology than those coated with polyvinyl alcohol. This is the basis for consideration when wanting to proceed to the finished formulation stage to determine the appropriate type of polymer, which indicates that during the process, there is no change from the results of the physical, chemical, and activity stability tests.

Microencapsulation technology in various products from various research results has shown enormous potential to maintain stability during processing and storage. Research and development efforts are still needed to identify and determine the type of coating that is adapted to the characteristics of the bioactive compounds as well as to improve and optimize the encapsulation method used to produce microcapsules that are physically, chemically, and activity stable.

CONCLUSION

The preparation of *S. oleosa* leaf extract microcapsules in fluid bed coating with polyvinyl alcohol and Ethocel 10 cP as a successful coating that can be used as a cover to protect bioactive chemicals from environmental changes during processing and storage The stability of biochemical compounds that are easily decomposed by external factors can be maintained by the microencapsulation method of *S. oleosa* leaf extract that we developed in this study. The microcapsules can maintain their total phenol content during

production, testing, and storage at various temperatures. This was further demonstrated by measuring total phenolic levels, assessing antioxidant activity *in vitro*, and proving hepatoprotective activity after stability testing gave consistent results.

ACKNOWLEDGMENT

The authors thank the Ministry of Education, Culture, Research, and Technology, Republic of Indonesia, for financially supporting this research in the Doctoral Dissertation Research Grant program of 2019-2021.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors contributed to data analysis, drafting or editing of the paper. They also approve the journal that will submit the article, provide final approval for the version to be published and agree to be responsible for all parts of the work.

CONFLICTS OF INTERESTS

The authors declare that they have no conflicts of interest.

REFERENCES

1. Muthukrishnan S, Sivakkumar T. Physicochemical evaluation, preliminary phytochemical investigation, fluorescence and TLC analysis of leaves of Schleichera oleosa (Lour.) Oken. Indian J Pharm Sci. 2018;80(3):525-32. doi: 10.4172/pharmaceutical-sciences.1000387.
2. Situmeang B, Nuraeni W, Ibrahim AM. Analysis of secondary metabolite compounds from leaves extract kesambi (Schleichera oleosa) and antioxidant activity test. J Pendidik Kim. 2018;8(Dec 2016):164-8.
3. Salem GA, Shaban A, Diab HA, Elsaghayer WA, Mjedib MD, Hnesh AM. Phoenix dactylifera protects against oxidative stress and hepatic injury induced by paracetamol intoxication in rats. Biomed Pharmacother. 2018;104:366-74. doi: 10.1016/j.biopha.2018.05.049, PMID 29778019.
4. Mbaoji FN, Nweze JA. Antioxidant and hepatoprotective potentials of active fractions of Lannea barteri Oliv. (Anarcadiaceae) in rats. Heliyon Heliyon. 2020;6(6):e04099. doi: 10.1016/j.heliyon.2020.e04099, PMID 32577550.
5. Sen S, Chakraborty R, Sridhar C, Reddy YSR, De B. Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect. Int J Pharm Sci Rev Res. 2010;3:91-100.
6. Nimse SB, Pal D. Free radicals, natural antioxidants, and their reaction mechanisms. RSC Adv. 2015;5(35):27986-8006. doi: 10.1039/C4RA13315C.
7. Thind TS, Singh R, Kaur R, Rampal G, Arora S. *In vitro* antiradical properties and total phenolic contents in methanol extract/fractions from bark of Schleichera oleosa (Lour.) Oken. Med Chem Res. 2011;20(2):254-60. doi: 10.1007/s00044-010-9297-2.
8. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. Hindawi Publishing; 2013. doi: 10.1155/2013/162750.
9. Zulham ZZ, Wilar G, Susilawati Y, Subarnas A, Chaerunisaa AY. Microparticles of herbal extracts with antioxidant activity. Pharmacogn J. 2021;13(1):285-95. doi: 10.5530/pj.2021.13.38.
10. Cabral BRP, de Oliveira PM, Gelfuso GM, Quintao TdSC, Chaker JA, Karnikowski MGdO. Improving stability of antioxidant compounds from Plinia cauliflora (jabuticaba) fruit peel extract by encapsulation in chitosan microparticles. J Food Eng. 2018;238:195-201. doi: 10.1016/j.jfoodeng.2018.06.004.
11. Muhaimin M, Latifah N, Chaerunisaa AY, Amalia E, Rostinawati T. Preparation and characterization of Sonneratia A. Leaf extract microcapsules by solvent evaporation technique. Int J App Pharm. 2022;14(6):77-82. doi: 10.22159/ijap.2022v14i6.46274.
12. Sukmawati A, Nurwaini S, Rahayu UB, Widawan APC, Safitri A, Astria NWN. Investigating betanin stability, release profile and antioxidant activity of ethyl cellulose microparticle containing beetroot (Beta vulgaris, linn) extract. Int J App Pharm. 2021;13(6):133-8. doi: 10.22159/ijap.2021v13i6.42848.
13. Li YO, Gonza D, Diosady LL. Microencapsulation of vitamins, minerals, and nutraceuticals for food applications; 2013. p. 501-22. doi: 10.1016/B978-0-12-404568-2.00038-8.
14. Lauro MR, Crasci L, Giannone V. An alginate/cyclodextrin spray drying matrix to improve shelf life and antioxidant efficiency of a blood orange by-product extract rich in polyphenols: MMPs inhibition and antiglycation activity in dysmetabolic diseases. Hindawi Publishing; 2017.
15. Ozkan G, Franco P, De Marco I, Xiao J, Capanoglu E. A review of microencapsulation methods for food antioxidants: principles, advantages, drawbacks and applications. Food Chem. 2019;272:494-506. doi: 10.1016/j.foodchem.2018.07.205, PMID 30309574.
16. Saikiran KCS, Perli M, Reddy NS, Venkatachalapathy N. Mechanical methods of microencapsulation: a review. Int J Curr Microbiol App Sci. 2018;7(11):1251-60. doi: 10.20546/ijcmas.2018.711.146.
17. Ballesteros LF, Ramirez MJ, Orrego CE, Teixeira JA, Mussatto SI. Encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds by freeze-drying and spray-drying using different coating materials. Food Chem. 2017;237:623-31. doi: 10.1016/j.foodchem.2017.05.142, PMID 28764044.
18. Ventura JM. Microencapsulation of ellagic acid from pomegranate husk and karaya. Int J Pharm Pharm Sci. 2015;7:10-3.
19. Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: a comprehensive review. Chin Med. 2018;13:20. doi: 10.1186/s13020-018-0177-x, PMID 29692864.
20. Sriwidodo S, Pratama R, Umar AK, Chaerunisa AY, Ambarwati AT, Wathoni N. Preparation of mangosteen peel extract microcapsules by fluidized bed spray-drying for tableting: improving the solubility and antioxidant stability. Antioxidants (Basel). 2022;11(7). doi: 10.3390/antiox11071331, PMID 35883823.
21. Blainski A, Lopes GC, De Mello JCP. Application and analysis of the folin ciocalteu method for the determination of the total phenolic content from Limonium brasiliense L. Molecules. 2013;18(6):6852-65. doi: 10.3390/molecules18066852, PMID 23752469.
22. Marinova G, Batchvarov V. Evaluation of the methods for determination of the free radical scavenging activity by DPPH. Bulg J Agric Sci. 2011;17(1):11-24.
23. Partial INF, Requirements THE, FOR D, Science MOF. Encapsulation of orange oil using fluidized bed granulation; 2019.
24. Murtaza G. Ethylcellulose microparticles: a review. Acta Pol Pharm. 2012;69(1):11-22. PMID 22574502.
25. Frey C. Fluid bed coating-based microencapsulation. Elsevier Inc; 2014. doi: 10.1016/B978-0-12-404568-2.00007-8.
26. Guignon B, Duquenoy A, Dumoulin ED. Fluid bed encapsulation of particles: principles and practice. Drying Technology. 2002;20(2):419-47. doi: 10.1081/DRT-120002550.
27. Hoyos leyva JD, Bello Perez LA, Alvarez Ramirez J, Garcia HS. Microencapsulation using starch as wall material: a review. Food Rev Int. 2018;34(2):148-61. doi: 10.1080/87559129.2016.1261298.
28. Wang R, Li M, Liu X, Sun Y. Preparation of composite fabric loaded with microencapsulated plant extracts and its inhibitory effect on lipase. PRT. 2019;48(3):202-9. doi: 10.1108/PRT-12-2017-0104.
29. Nazzaro F, Orlando P, Fratianni F, Coppola R. Microencapsulation in food science and biotechnology. Curr Opin Biotechnol. 2012;23(2):182-6. doi: 10.1016/j.copbio.2011.10.001, PMID 22024623.
30. Muhaimin M, Yusnaidar Y, Syahri W, Latief M, Chaerunisaa AY. Microencapsulation of macaranga gigantea leaf extracts: production and characterization. PJ. 2020;12(4):716-24. doi: 10.5530/pj.2020.12.104.
31. Khan H, Ullah H, Nabavi SM. Mechanistic insights of hepatoprotective effects of curcumin: therapeutic updates and future prospects. Food Chem Toxicol. 2019;124:182-91. doi: 10.1016/j.fct.2018.12.002, PMID 30529260.

32. Rotundo L, Pyrsopoulos N. Liver injury induced by paracetamol and challenges associated with intentional and unintentional use. World J Hepatol. 2020;12(4):125-36. doi: 10.4254/wjh.v12.i4.125, PMID 32685105.
33. Mirza N. Paracetamol-induced hepatotoxicity, edited: Costin Teodor Streba CT, Rogoveanu I, Vere CC. Vol 11; 2022. doi: 10.5772/intechopen.104729.
34. Katiyar NS, Singh AP, Gangwar AK, Rao NV. Evaluation of hepatoprotective activity of stem extracts of Cuscuta reflexa (ROXB) in rats. Int J Pharm Pharm Sci. 2015;7(6):231-4.
35. Jajo H, Ghosh R. Hepatoprotective activity of the whole plant of Neptunia prostrata L. in carbon tetrachloride induced rats. Int J Curr Pharm Sci. 2021;13(6):56-9. doi: 10.22159/ijcpr.2021v13i6.1913.