INTRODUCTION

Herbal extracts are an important source of biologically active compounds with a central role in human health. According to the available data, now days 80% of the world’s population uses herbs and herbal medicines for the treatment of various ailments. The acceptability of herbal formulations is increasing due to their affordability, effectiveness and low toxicity. Herbal extracts have been widely accepted as the potential medicines with less side effects as compared to synthetic drug molecules [1-3]. In recent years, focus has been directed towards the development of drug delivery system using biologically active compounds derived from natural sources [1, 2]. 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 [4-6]. Application of valuable natural compounds can be limited by their unpleasant taste, low bioavailability, volatilization of active compounds, sensitivity to the temperature, oxidation and UV light, as well as in vivo instability [3]. One of the potential strategies to overcome these issues is microencapsulation of the herbal active ingredients. Therefore, microencapsulation technique can be used for obtaining herbal products with desirable characteristics. Microencapsulation techniques of plant materials and other natural products are widely used in the food, pharmaceutical and cosmetic industries [3, 5]. 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 [7-15].

*Prema serratifolia* lam is an important plant belonging to the family Verbenaceae and is one of the most widespread large shrubs in the forests of Indonesia. The whole plant possesses medicinal properties. *P. serratifolia* (bebuah) is a tropical and subtropical medicinal herb that is widely spread and frequently utilized in the traditional system of medicine. *P. serratifolia* is a thorny, erect shrub or small tree with a scendent stem and big branches [16-22]. The leaves are either opposite or whorled and they are either whole or serrate. *P. serratifolia* has a lot of therapeutic usefulness and it’s been studied a lot [5-8].

Solvent evaporation method is a popular technique for the encapsulation of drugs within polymeric microcapsules. Water-insoluble polymers are usually used as encapsulation matrix for these microcapsules. Microcapsules have been prepared with a wide range of polymers and polymer blends. The solidification rate of polymeric microcapsules is an important parameter influencing the particle size,
the encapsulation efficiency and the initial burst in microparticulate systems. Various types of polymer with different physical properties (such as biodegradable, non-biodegradable, permeable, etc.) have been prepared as microcapsules. They are Eudragit® E100 and ethyl cellulose microspheres. Eudragit® E100 is a cationic copolymer based on dimethylamino ethyl methacrylate, butyl methacrylate, and methyl methacrylate with a ratio of 2:1:1 [7]. The monomers are randomly distributed along the copolymer chain. Eudragit E100 are targeted for immediate drug release. While, ethyl cellulose is a nonbiodegradable hydrophobic polymer. Ethylcellulose is one of the most commonly used polymer for sustained release and develop extended-release multiparticulate dosage forms, but is also used for moisture protection and taste masking. It is insoluble but permeable in water and guarantees a pH-independent drug release [14, 23].

Microencapsulation is a process by which solids, liquids or gases are surrounded with a membrane or matrix [3]. Solvent evaporation method has been widely and extensively used to prepare polymeric microspheres containing different drugs and in the development of modified release systems [23-28]. 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 [29-34]. It is an instantaneous process where spherical and uniform samples can be obtained, and the process can be easily scaled up [34-39]. The technique of microencapsulation by solvent evaporation is widely applied in the pharmaceutical industry to obtain the controlled release of drug. The obtained polymer microspheres with drug trapped inside can degrade and release the encapsulated drug slowly with a specific release profile. This controlled drug release has outstanding clinical benefits: reducing of dosing frequency, more convenience and acceptance for patients, and drug targeting to specific locations resulting in a higher efficiency [40-45]. There are different methods to use microencapsulation by solvent evaporation technique. The choice of the method that will give rise to an efficient drug encapsulation depends on the hydrophilicity or the hydrophobicity of drug. For insoluble or poorly water-soluble drugs, the oil-in-water (o/w) method is frequently used. This method is the simplest and the other methods derive from this one [3, 32, 39, 46-51]. Another alternative to encapsulate hydrophilic drugs is to employ the water-in-oil-in-water (W/O/W) emulsion process. The study included the preparation and evaluation of the microcapsules obtained P. serratulifolia leaves extract using the solvent evaporation method.

MATERIALS AND METHODS
Collection of plant materials
Fresh leaves of P. serratulifolia were collected in the month of January 2022 from Jambi and identified by a taxonomist from the Department of Biology, Faculty of Mathematics and Natural Sciences of Padjadjaran University. A reference specimen (No. 79/IBM/IT/7/2022) was securely archived for future reference.

Chemicals and reagents
Quercetin dehydrate, Ethanol 10 cp, Eudragit E 100, glacial acid, ethanol (CH3OH), methanol, dichloromethane, hydrochloric acid, sodium hydroxide, PVA were bought from Merck Chemicals GmbH, Darmstadt, Germany. Naphthylethenediamine dihydrochloride (PanReac AppChem, Darmstadt, Germany), Thiobarbituric acid (TBA) and Trichloro acetic acid (Sigma-Aldrich, St. Louis, MO, USA). All other reagents used were of analytical grade and were purchased from Sigma-Aldrich (Saint Louis, MO, USA) and PanReac AppChim (Darmstadt, Germany). UV spectra were recorded in Shimadzu 1601 UV-Visible spectrophotometer. The chemicals used were of good quality and quantity standard and do not require further purification.

Extraction
Dried P. serratulifolia leaves (3 kg) were graded and extracted three times with 30 l of ethanol (24 h each) by maceration technique. The macerate was then concentrated, evaporated and dried in a vacuum at 60 °C using a rotary evaporator (buchi rotovap R-205). The yield value was as much as 25.3% (w/w). The dry extract was stored in refrigerator at 4 °C until when it will be used.

Preparation of polymeric microspheres
The solvent evaporation method based on the formation of W/O emulsions used to prepare microspheres using 10% ethyl cellulose [Ethocel 10 cp (E100) (10%, 15%, 20%)] were dissolved in 20 ml dichloromethane. 5 g of P. serratulifolia leaves extract were dissolved within this organic phase. The organic phase was then emulsified into 800 ml aqueous PVA solution (1.5% w/v) containing 0.5 M NaCl and NaOH at pH 12. The emulsion was stirred for 1 h at 500 rpm with a propeller sturrer (Heidolph Elektro GmbH and Co. KG, Kelheim, Germany) to allow microsphere hardening. After 4 h, the microspheres 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 [7, 10].

Particle size analysis
Particle size mean and size distribution of the microspheres 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 polydispersity of the resulting heterogeneous suspensions were determined using the DLS technique. The microcapsules 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 P. serratulifolia leaves, and microcapsule with formula 20% ethocel 10 cp (EA2) and formula 20% Eudragit E100 (EB2) 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 microspheres was analysed by scanning electron microscopy (SEM). For surface imaging, the microspheres 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
Microspheres (10 mg) were extracted in 1 ml methanol, followed by agitation in a horizontal shaker (IKA HS 501 digital horizontal Shaker, Janke and Kunkel GmbH and Co. KG IKA labotechnik, 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).

The compound used as a standard in determination of flavonoids level was quercetin, since quercetin is a flavonoid class of flavonol that has a keto group at atom C-4 and also the hydroxyl groups of atoms C-3 and C-5, which neighbors. Determination of the maximum quercetin wavelength was done by running quercetin solvent at a wavelength range of 400 nm, 405 nm, 410 nm, 415 nm, 420 nm, 425 nm, 430 nm, 435 nm, 440 nm and 450 nm. The results indicated that the maximum wavelength of quercetin was 435 nm, Flavonoid (in P. serratulifolia leaves extract) concentration in the obtained aqueous solution was determined by UV-spectrophotometry at wavelengths of 435 nm, respectively (HP 8453 UV-Vis spectrophotometer, Agilent Technologies Deutschland GmbH, Waldbronn, Germany). The actual drug loading and encapsulation efficiency were calculated as follows [21]:

Actual drug loading (%) = (Moad/Mox) x 100% ———— (1)
Encapsulation Efficiency (%) = (Moad/Mox) x 100% ———— (2)
where \(M_{act}\) = actual flavonoid (quercetin) content in weighed quantity of microparticles, \(M_{the}\) = weighed quantity of microparticles and \(M_{iso}\) = theoretical flavonoid (quercetin) content in microparticles.

**RESULTS AND DISCUSSION**

**Microparticle**

Microencapsulation by solvent evaporation technique is widely used in pharmaceutical industries. It facilitates a controlled release of a drug which has many clinical benefits. Water insoluble polymers are used as encapsulation matrix using this technique [23, 24, 34]. For insoluble or poorly water-soluble drugs such as *P. serratfolia* leaves extract, an O/W method is suitable used [34].

The controlled release of drug in pharmaceutical applications can be achieved by the microencapsulation by solvent evaporation technique. The properties of materials and the process engineering aspects strongly influence the properties of microspheres and the resultant controlled release rate. 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 was 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 [30-35].

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 kinds of polymers, including Ethocel 10 cP and Eudragit E100. Ethyl cellulose (EC) is a partly O-ethylated cellulose ether derivative. It is available in a variety of grades, which differ in viscosity, is 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 often used in the coating process in the pharmaceutical field [7, 24].

Eudragit E100 is a cationic polymer based on dimethyldinoethyl methacrylate, butyl methacrylate, and methyl methacrylate. The glass transition temperature of Eudragit E100 is ~ 46 °C. If it 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 [7].

Leaves of *P. serratfolia* contains secondary metabolite compounds such as flavonoids. The content of flavonoids in leaves was determined using a standard curve of quercetin. The analysis of maximum wavelength absorption indicated that the maximum wavelength standards of quercetin was at a wavelength of 435 nm [7, 15, 17]. Absorbance measurement of quercetin standard solvent obtained was used to get a calibration curve of standard solvent of quercetin such as concentration curve graph versus absorbance. The results of the calibration curve of standard solvent of the compound quercetin was obtained linear relationship between the absorbance with concentration. Standard curve of quercetin at a wavelength of 435 nm maximum was used for determination flavonoids content in microparticles or encapsulation efficiency.

**Table 1: Data of microparticles from ethanol extract of *P. serratfolia* leaves with homogenizer speed of 2,700 rpm (5 min) followed by stirring by propeller stirrer 500 rpm (4 h)**

<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>EA₁</td>
<td>Extract 5 g Ethocel 10 cP = 10% PVA = 1.5%</td>
<td>4.584</td>
<td>1.127±0.29</td>
<td>81.412±3.21</td>
</tr>
<tr>
<td>EA₂</td>
<td>Extract 5 g Ethocel 10 cP = 15% PVA = 1.5%</td>
<td>4.736</td>
<td>1.583±0.46</td>
<td>84.106±4.34</td>
</tr>
<tr>
<td>EA₃</td>
<td>Extract 5 g Ethocel 10 cP = 20% PVA = 1.5%</td>
<td>4.815</td>
<td>1.662±0.38</td>
<td>85.574±3.72</td>
</tr>
<tr>
<td>EB₁</td>
<td>Extract 5 g Eudragit E100 = 10% PVA = 1.5%</td>
<td>4.221</td>
<td>1.024±0.37</td>
<td>80.632±5.41</td>
</tr>
<tr>
<td>EB₂</td>
<td>Extract 5 g Eudragit E100 = 15% PVA = 1.5%</td>
<td>4.285</td>
<td>1.283±0.72</td>
<td>83.004±3.85</td>
</tr>
<tr>
<td>EB₃</td>
<td>Extract 5 g Eudragit E100 = 20% PVA = 1.5%</td>
<td>4.473</td>
<td>1.491±0.56</td>
<td>83.102±5.47</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD, n=3

**Annotation**

EA₁ = Formula with ethocel 10 cP (10%)

EA₂ = Formula with ethocel 10 cP (15%)

EA₃ = Formula with ethocel 10 cP (20%)

EB₁ = Formula with eudragit E100 (10%)

EB₂ = Formula with eudragit E100 (15%)

EB₃ = Formula with eudragit E100 (20%)

PVA = Polivinyl Alkalohol

PSA = Particle Size Analyzer

EE = Encapsulation efficiency

Ethanol extract of *P. serratfolia* has low stability because it contains natural ingredients, so it is formulated in the form of microparticles by utilizing a polymer that can protect the extract as an active ingredient. The polymer used is ethocel 10 cP and eudragit E100 with a concentration variation of 10, 15 and 20%, respectively. PVA in microparticles preparations is commonly used as a polymer stabilizing agent in the solvent evaporation method. However, the use of the polymer must be able to guarantee the stability of the extract, especially in terms of activity [7, 14, 22].

Based on observations of microparticles from ethanol extract of *P. serratfolia* leaves using polymer variations and different concentration variations above, we have obtained yields of each polymer with different concentrations, i.e at 10% ethocel 10 cP produces 4.584 g besides that 15% ethocel 10 cP produces 4.736 g while the 20% ethocel 10 cP produces 4.815 g (table 1). Likewise, with...
the eudragit E100 polymer with various concentrations, 10% eudragit E100 produces 4.221 g, in addition to the 15% eudragit E100 produces 4.285 g, while the 20% eudragit E100 produces 4.473 g (table 1). It can be concluded that the higher the concentration used in ethocel 10 CP and eudragit E100 polymers, the higher the yield obtained.

Based on the results of the characterization of the microsphere particle size of the P. serratulfolia leaf extract microparticles in a formula using a 10% ethocel 10 CP polymer resulting in a particle size of 1.127 µm, when to formulas that use a 15% ethocel 10 CP polymer particle size of 1.583 µm, whereas in formulas with 20% ethocel 10 CP polymer particle size of 1.662 µm.

From the analysis data shows that the particle size is categorized into a micro size that is above 1 µm. From 3 variations of the concentration of ethocel 10 CP and eudragit E100 polymers, it can be seen that at a concentration of 20% shows the largest particle size compared to the other concentration (table 1). But these particle size is still categorized into a micro size that is above 1 µm.

Next is calculating the value of % encapsulation efficiency (% EE), which aims to find out how much % of the ethanol extract P. serratulfolia leaves used can be coated by polymer. In the formula using 10% ethocel 10 CP polymer, it is known that the % EE value is 81.412%. Using 15% ethocel 10 CP polymer is known that the % EE value is known as 84.106%, while the 20% ethocel 10 CP polymer is known to be the % EE value of 85.574%. It is categorized as good because it has the % EE value of ≥ 80%.

Whereas in formulas that use 10% eudragit E100 polymer, the % EE value is known to be 60.632%, the 15% eudragit E100 polymer give the % EE value is known to be 83.004%, while at a concentration of 20% eudragit E100 polymer give the % EE value is known to be 83.102%. It is also categorized as good because it has the % EE value of ≥ 80%.

Zeta potential

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 30 mV or less than-30 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 [37-42]. 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.

Zeta potential value of P. serratulfolia leaves ethanol extract microcapsule (EA₃ = Ethocel Formula 10 CP (20%)) that values-19.8 mV (fig 1a), while zeta potential value of P. serratulfolia leaves ethanol extract microcapsule (EB₃ = Eudragit E100 Formula (20%)) that values-15.7 mV (fig. 1b). This shows that the two polymers used in preparation of these microparticles produce dispersions with low zeta potential values that will produce aggregates due to inter-particle Van Der Waals attractions.

Zeta potential is the electrical potential at the slipping plane. This plane is the interface which separates mobile fluid from the fluid that remains attached to the surface. Zeta potential is a scientific term for electrophoretic potential in colloidal dispersions. In the colloidal chemistry literature, it is usually denoted using the Greek letter zeta (ζ), hence ζ-potential. The usual units are volts (V) or, more commonly, millivolts (mV). From a theoretical viewpoint, the zeta potential is the electric potential in the interfacial double layer (DL) at the location of the slipping plane relative to a point in the bulk fluid away from the interface. In other words, zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle [7, 14, 22].

The zeta potential is caused by the net electrical charge contained within the region bounded by the slipping plane and also depends on the location of that plane. Thus, it is widely used for quantification of the magnitude of the charge. However, zeta potential is not equal to the Stern potential or electric surface potential in the double layer because these are defined at different locations. Such assumptions of equality should be applied with caution. Nevertheless, zeta potential is often the only available path for characterization of double-layer properties.

Zeta potential can also be used for the pKa estimation of complex polymers that is otherwise difficult to measure accurately using conventional methods. This can help studying the ionisation behaviour of various synthetic and natural polymers under various conditions and can help in establishing standardised dissolution-pH thresholds for pH-responsive polymers.

Thin layer chromatography

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 [7].

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Fig. 1: Zeta potential profile of P. serratulfolia leaves ethanol extract microparticles [(a) EA₃ = Ethocel formula 10 CP (20%); (b) EB₃ = Eudragit E100 formula (20%)]

![Fig. 1: Zeta potential profile of P. serratulfolia leaves ethanol extract microparticles](image)

Fig. 2: TLC profile of P. serratulfolia leaves ethanol extract; EA₃ = Ethocel formula 10 CP (20%); EB₃ = Eudragit E100 formula (20%) under UV light 366 nm

The results obtained from the ethanol extract of P. serratulfolia leaves seen under UV light 366 nm showed the presence of four and five stains with varying Rf values (fig 2 and table 2).
It can be seen from the results of TLC above (fig. 2) shows that the presence of polymers with certain concentrations does not affect the compounds contained in the ethanol extract of *P. serratifolia* leaves. So it can be concluded that formula with 20% ethocel 10 cP (EA3) and 20% E100 Eudragit polymer (EB3) produce a stable formula. This is shown by the results of the spots on the TLC, which are evident when compared to the extract but also are considered based on the results of the particle size and the value of % EE that is better than other formulas.

### Scanning electron microscope

Microparticles refer to particles with a diameter of 1-1000 μm. Microparticles are usually assumed that the formulations described as Micro particles consist of active substances and polymer mixtures. After scanning electron microscope can see the microparticles using ethocel 10 cP and Eudragit E100 polymers, namely microparticles using ethocel 10 cP has a smoother surface and less visible pores (fig. 3. a1 and b1), while microcapsules using Eudragit E100 polymers have a rough and porous surface (fig. 3. a2 and b2). Microcapsules using Ethocel 10 cP have a particle size of 1.662 μm (EA3) and using Eudragit E100 have a particle size of 1.491 μm (EB3), this indicates that the resulting particle size has met the size requirements of the microparticles [7, 10, 21]. Polymer type used on this study is applicable to produce microparticles.

### ACKNOWLEDGEMENT

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### FUNDING

Nil

### AUTHORS CONTRIBUTIONS

Conceptualization: M. M, S. N, Y. I and W. S; Methodology: M. M, S. N, Y. I and W. S; Data curation: M. M, S. N, Y. I and A. H.; Writing—Original Draft Preparation: M. A, A. H and R. H.; Writing—Review and Editing: M. M, A. H and R. H; Supervision: S. N, M. M and Y. I; Funding Acquisition: M. M; All authors have read and approved the final manuscript.

### CONFLICTS OF INTERESTS

No conflicts of interest is associated with this work.

### REFERENCES


### Table 2: Rf value calculation results in thin layer chromatography profiles

<table>
<thead>
<tr>
<th>Rf Value</th>
<th>Extract</th>
<th>EA3</th>
<th>EB3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.67</td>
<td>0.67</td>
<td>0.68</td>
</tr>
<tr>
<td>2</td>
<td>0.62</td>
<td>0.62</td>
<td>0.63</td>
</tr>
<tr>
<td>3</td>
<td>0.49</td>
<td>0.49</td>
<td>0.50</td>
</tr>
<tr>
<td>4</td>
<td>0.37</td>
<td>0.37</td>
<td>0.37</td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
<td>0.26</td>
<td>0.26</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>0.18</td>
<td>0.18</td>
</tr>
</tbody>
</table>

CONCLUSION

Based on the result, it concluded that microparticles of *P. serratifolia* leaves extract can be prepared by solvent evaporation method with single emulsion system (O/W: oil in water) using Eudragit E100 and Ethocel 10 cP as polymer. Characterization of the microparticles revealed that polymer type used on this method is applicable to produce microparticles.


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