Nanoencapsulation of *G. procumbens* with polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP) is intended to increase anti-dengue activity and prevent its bioactive deterioration. The particle size of Ga-NPs was 161.00 ± 2.17 nm with a PDI value of 0.33 ± 0.02 while the particle size of Gp-NPs was 136.33 ± 0.47 nm with a PDI value of 0.34 ± 0.01. Those indicated both Ga-NPs and Gp-NPs had a homogeneous particle size distribution. Moreover, both nanocapsules showed stability at 30-100°C but were less stable at high pH and salt concentrations. Ga-NPs have a %LE (96.236 ± 1.156%) higher than the %LE of Gp-NPs (95.567 ± 0.334 %), but release of Gp-NPs is better than Ga-NPs. *G. procumbens* extract, Ga-NPs, and Gp-NPs exhibited anti-dengue activity with IC\textsubscript{50} values of 11.22, 7.67, and 12.75 µg/mL, respectively. However, Ga-NPs and Gp-NPs are more potent because they have higher SI values with low cytotoxicity (CC\textsubscript{50}) compared to *G. procumbens* extract. Herein, nanocapsulation of *G. procumbens* is proved to increase bioavailability and controlled release and allow precision targeting of bioactive compounds to enhance bioactivity activity.

**Keywords:** Anti-dengue, *Gynura procumbens*, Nanoencapsulation, Polyvinyl Alcohol, Polyvinylpyrrolidone.

**INTRODUCTION**

*Gynura procumbens* is one of the medicinal plants found in China, Vietnam, Indonesia, Malaysia, and Thailand.\(^1\) *G. procumbens* contains various secondary metabolites, such as quercetin, gallic acid, kaempferol, kaempferol-3-O-β-D-glucopyranoside, kaempferol-3-O-rutinoside, rutin, chlorogenic acid, 3,5-dicafeoylquinate methyl ester, terpenoids, tannins, alkaloids, saponins, and astragali.\(^2,3\) In addition, *G. procumbens* contains several phenolic acid compounds, such as gallic acid, protocatechuic acid, vanillic acid, syringic acid, caffeic acid, p-coumaric acid, ferulic acid, and synaptic acid.\(^4\) *G. procumbens* is reported to have various biological activities, including antioxidants\(^5,6\), antidiabetic\(^7\), antimicrobial\(^8,9\), anticancer\(^10\), and anti-inflammatory.\(^9\) There is no study that reported the activity of *G. procumbens* against dengue infection. *G. pseudo china*, species from the same genus as *G. procumbens*, showed anti-dengue by increasing the number of platelets in mice at a dose of 500 mg/kg BW.\(^11\) Therefore, it is expected that *G. procumbens* also has anti-dengue activity. The incorporation of herbal medicine with nanotechnology is a breakthrough in drug development. Nanoencapsulation is a nanotechnology application that can increase protection and bioavailability of bioactive compounds because the nano size can increase the available

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surface area.\textsuperscript{12} Additionally, the coating process of a bioactive compound at the nano size maintains its stability.\textsuperscript{13} In general, nanoencapsulation can enhance the biological activity of herbal medicine. This study aims to make \textit{G. procumbens} nanocapsules through the nanoencapsulation process of \textit{G. procumbens} extract with different polymers which then assessed for its anti-dengue activity. In particular, nanoencapsulation is expected to increase the anti-dengue activity of \textit{G. procumbens} extract.

**EXPERIMENTAL**

**Materials**

Methanol, polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), distillate water, HCl, NaCl, NaOH, buffer pH 2, 7, and 8.5, dimethyl sulfoxide (Merch), fetal bovine serum, Vero cell line (African green monkey), fungizone (Sigma), Viral ToxGloTM assay reagents (Promega), CellTiter-Glo® Luminescent Cell Viability Assay (Promega), Minimum essential medium eagle (MEM), 3-(4,5)-dimethylthiazole-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), Trypan-EDTA, Trypan blue, DENV-2 (NCBI No. KT012513, Dengue Study Group, Institute of Tropical Disease) Universitas Airlangga.

**G. procumbens Preparation**

The \textit{G. procumbens} leaves were obtained from Mount Lawu, Jogorogo, Ngawi, East Java, Indonesia. \textit{G. procumbens} dried powder (1 kg) was extracted with methanol solvent at 1: 3 (w/v) for 1 x 24 hours. The methanol extract of \textit{G. procumbens} was concentrated using a rotary vacuum evaporator. The components of secondary metabolites from the methanol extract of \textit{G. procumbens} were identified using LC-MS.

**G. procumbens Nanoencapsulation**

The manufacture of \textit{G. procumbens} nanoencapsulation refers to Aminah \textit{et al.} \textsuperscript{202.14} The Ga-NPs and Ga-NPs were nano \textit{G. procumbens} with PVA and PVP, respectively. The physicochemical properties of both nano \textit{G. procumbens} consisted of particle size, Zeta potential, and polydispersity index (PDI) that were measured using DLS (Zetasizer Nano ZS, Malvern). The functional groups of the nano \textit{G. procumbens} were determined by FTIR (Shimadzu IRTracer-100). The decomposition of nano \textit{G. procumbens} was determined using TGA at 25-900 °C. Meanwhile, the topology of both nano \textit{G. procumbens} was analyzed using AFM (Bruker, Nanoscan).

**Stability, Loading, and Release**

Ga-NPs and Gp-NPs were evaluated their stability based on changes in UV-Vis spectra and turbidity levels on alteration of temperature, pH, and NaCl concentration.\textsuperscript{15} The levels of \textit{G. procumbens} (gallic acid and quercetin) bioactive components in Ga-NPs and Gp-NPs were determined using a UV-Vis spectrometer (Shimadzu UV-1800). The loading efficiency and amount of the two nano \textit{G. procumbens} are determined based on Eqns. 1 and 2.\textsuperscript{16} Meanwhile, the release of bioactive components of both nano \textit{G. procumbens} was determined at pH 2, 7, and 8.5, which is the pH condition of the digestive system.

\[
%LE = \frac{\text{Mass of samples on G-NPs}}{\text{Mass of samples in feed}} \times 100\% \quad (1)
\]

\[
%LA = \frac{\text{Mass of samples on G-NPs}}{\text{Mass of G-NPs}} \times 100\% \quad (2)
\]

**Anti-dengue Assay**

Anti-dengue assay of \textit{G. procumbens} extract, Ga-NPs, and Gp-NPs was performed by Viral ToxGlo assay. Vero cells (ATCC® CCL-81TM) were seeded in a 96-bottom luminescence microplate which was then treated with 25 µL various concentrations of samples. Consequently, DENV-2 stock (KT012509) Surabaya isolates with a concentration of 2 x 10^3 FFU/mL were added with a volume of 25 µL. All treatments were incubated for 48-144 hours in an incubator (37°C; 5% CO\textsubscript{2}). As much as 100 µL of Viral ToxGlo assay reagent was added upon incubation. Furthermore, incubation was carried out for 10 minutes in an incubator (37°C, 5% CO\textsubscript{2}). The absorbance of each sample was read using a GloMax reader on the luminescence.
menu, which was further used to calculate IC\textsubscript{50} and CC\textsubscript{50}. The selectivity index (SI) was obtained by dividing the given IC\textsubscript{50} into CC\textsubscript{50}.

RESULTS AND DISCUSSION

Phytochemical Profiling
The phytochemical profile of the methanolic extract of \textit{G. procumbens} aims to determine the secondary metabolite components contained in \textit{G. procumbens}. Phytochemical profiling is performed by using LC-MS. It provides information in the form of molecular weight, structure, and quantity of secondary metabolites in the sample. The result shows that \textit{G. procumbens} extract contained 62 secondary metabolites. These compounds belong to the phenylpropanoid group, alkaloids, terpenoids, steroids, flavonoids, lipids, and benzoic acid derivatives.

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)
Total Phenolic Content (TPC) is used to determine the content of phenolic compounds in the extract of \textit{G. procumbens}. The TPC value is expressed in mg gallic acid equivalent per gram (mg GAE/g).\textsuperscript{17} The phenolic content in the extract has 10.492 ± 0.025 (mg GAE/g). Meanwhile, Total Flavonoid Content (TFC) is used to determine the flavonoid content in the extract of \textit{G. procumbens}. The TFC value is expressed in mg quercetin equivalents per gram (mg QE/g).\textsuperscript{17} The flavonoid content in the extract of \textit{G. procumbens} is 0.320 ± 0.073 (mg QE/g).

Quercetin and Gallic Acid Contents
Based on the results of LC-MS analysis, the content of gallic acid and quercetin in the extract of \textit{G. procumbens} is higher than other secondary metabolites. Therefore, in this study, gallic acid and quercetin contents were determined in the extract. Based on the calculation results, gallic acid and quercetin levels are 42.41 ± 0.96 and 34.76 ± 1.25 mg/g extract, respectively.

Characterization of Nano \textit{G. procumbens}
The characterization of nano \textit{G. procumbens} in physicochemical properties consisted of particle size, polydispersity index (PDI), and Zeta potential (ζ), as shown in Table-1. Particle size ensures that each sample is classified as a nanoparticle. Nanoparticles are colloid-sized particles that have a maximum diameter of 500 nm.\textsuperscript{18} The particle sizes of Ga-NPs and Gp-NPs are 161.00 ± 2.17 and 136.33 ± 0.47 nm, respectively. It shows that the nanoencapsulation process of \textit{G. procumbens} extract with PVA and PVP meets the requirements of nanoparticles.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ga-NPs</th>
<th>Gp-NPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size ± SD (nm)</td>
<td>161.00 ± 2.17</td>
<td>136.33 ± 0.47</td>
</tr>
<tr>
<td>PDI ± SD</td>
<td>0.33 ± 0.02</td>
<td>0.66 ± 0.02</td>
</tr>
<tr>
<td>ζ ± SD (mV)</td>
<td>-15.80 ± 1.87</td>
<td>-22.63 ± 1.31</td>
</tr>
</tbody>
</table>

The polydispersity index (PDI) measures sample heterogeneity based on particle size caused by size distribution, aggregation, and agglomeration in the sample. Table-1 shows the PDI of Ga-NPs and Gp-NPs of 0.33 ± 0.02 and 0.34 ± 0.01, respectively. These results indicate that the size distribution of Ga-NPs and Gp-NPs tends to be homogeneous. PDI value less than 0.05 (mono dispersion sample) indicates a narrow particle size distribution so that the particle size is uniform (homogeneous). Meanwhile, a PDI value of more than 0.7 indicates an extensive particle size distribution, so the particle size is not uniform.\textsuperscript{19}

Table-1: Physiochemical Properties of Nano \textit{G. procumbens}.

Fig.-1: FTIR Spectrums of (A) Ga-NPs and (B) Gp-NPs
Zeta potential (ζ) is a parameter of electric charge on the surface of nanoparticles. A good zeta potential value indicates that the strength of the particles repelling each other is getting more vital to produce a stable sample dispersion. Particles with a zeta potential value of less than ±30 mV are considered stable. Table-1 shows the zeta potential value (ζ) of the Ga-NPs and Gp-NPs of -15.80 ± 1.87 and -22.63 ± 1.31 mV, respectively. The Ga-NPs and Gp-NPs have a zeta potential value less than ±30 mV, indicating that both nano G. procumbens were stable and not easy to aggregate. Characterization of Ga-NPs by FTIR shows several absorption bands in the 3322 cm⁻¹ regions, which indicate overlapping -OH groups of G. procumbens extract and PVA (Fig.-1). The absorption bands at 1571 and 1250 cm⁻¹ are the characteristics of C=C in the ring and C-O stretching groups from G. procumbens extract. The absorption bands at 1250 and 1074 cm⁻¹ are the C-H bending region and the secondary alcohol group of PVA.

![Figure 2: Thermogram of (A) Ga-NPs and (B) Gp-NPs](image)

The Gp-NPs show some typical absorption bands. The absorption band 2925 cm⁻¹ is the C-H sp³ group. Several absorption bands of G. procumbens extract appeared at 1370 and 1167 cm⁻¹, which indicated the C-H bending and C-O stretching groups of the G. procumbens extract. Meanwhile, the PVP characteristic appears in 1640, 1433, and 1286 cm⁻¹ regions, which are C=O, C-H bending, and C-N stretching groups (Fig.-1). TGA Ga-NPs analysis reveals a decrease in weight at a temperature of 89°C, which indicate the release of water molecules by 34% (Fig.-2). Weight loss also occurs at a temperature of 330°C, indicating the decomposition of G. procumbens extract components by 31%. Meanwhile, weight loss of 18% at a temperature of 487°C is the decomposition of PVA. The decomposition of Gp-NPs at a temperature of 104°C results in a weight loss of 22%, considering a release of water molecules (Fig.-2). The weight loss of 57% at 412°C is the decomposition of PVP. Meanwhile, a 10% decrease of weight at 679°C is considered the decomposition of G. procumbens extract in Gp-NPs. AFM analysis shows the surface topology of nano G. procumbens. The particle size of Ga-NPs and Gp-NPS are ±150 nm and ±100 nm, respectively. It shows that the particle size of the AFM results is close to the DLS measurement (Fig.-3).

![Figure 3: Topography of (A) Ga-NPs and (B) Gp-NPs](image)

**Stability of Nano G. procumbens**

**Stability on Temperature**

The stability of Ga-NPs and Gp-NPs subject to different temperatures was determined by heating them at a temperature of 30-100°C. Furthermore, the measurement of the turbidity level in both nano G. procumbens was performed to analyze the level of stability. The turbidity level of G. procumbens extract, Ga-NPs, and
Gp-NPs tends to be stable at all increasing temperatures. The turbidity level of *G. procumbens* extract is higher than Ga-NPs and Gp-NPs. It is because the active compounds contained in *G. procumbens* are directly exposed to temperature during the heating process, so they are more easily degraded. Ga-NPs and Gp-NPs are relatively more stable and not easily degraded in the heating process because the active compounds of *G. procumbens* are protected by polymers. In addition, PVA and PVP used in Ga-NPs and Gp-NPs are stable when heated to 100°C. The turbidity level concerning temperature is shown in Fig.-4. The heating factor does not affect the stability of the bioactive components in the *G. procumbens* extract, Ga-NPs, and Gp-NPs. Moreover, the extract and both nano *G. procumbens* have two absorption bands at 615 and 408 nm. The heating treatment does not result in a shift or change in the spectral pattern of the three absorption bands (Fig.-5).

**Stability on pH**

The pH stability was measured to determine the stability level of *G. procumbens* extract, Ga-NPs, and Gp-NPs at pH 2-11. The turbidity level of the extract and nano *G. procumbens* affected by pH is shown in Fig.-4. The base pH of the three samples is pH 6. When the pH is acidified to pH 5, the extract and both nano *G. procumbens* show an increase. In *G. procumbens* extract (pH 4), the turbidity level decreased and rose again at pH 2 and 3. In Ga-NPs and Gp-NPs (pH 4), the turbidity level decreased and was relatively stable at pH 2-3. The decrease in turbidity level also occurred at alkaline pH for both extract and nano *G. procumbens*. The turbidity level of *G. procumbens* extracts tends to be higher at each pH level than the two nano *G. procumbens*. The UV-Vis spectra of the *G. procumbens* extract convey that pH 6 has two absorption bands at 614 and 408 nm. The hyperchromic shift of band II shows at alkaline pH (8-11), while a shift in bands I and II happened at acidic pH (2-4). Moreover, an absorption band appears at 325 nm in acidic pH. The Ga-NPs and Gp-NPs are relatively stable at pH 6-11. However, at acidic pH, there is a slight hyperchromic or hypochromic shift in the absorption band (Fig.-6).

**Stability on NaCl**

The stability of *G. procumbens* extracts, Ga-NPs, and Gp-NPs concerning salt concentration is carried out by increasing the concentration of 0 - 0.3 M (Fig.-4). The increase in NaCl concentration causes an increment in the turbidity level of *G. procumbens*. Initially, there was a significant increase in turbidity of
Ga-NPs and Gp-NPs treated with a NaCl concentration of 0 - 0.1 M. In contrast, the turbidity level continued to decrease significantly upon being treated with NaCl 0.15 - 0.3 M. The decrease in turbidity is due to the precipitation process. An increase in turbidity and the formation of a precipitate in each sample indicates that *G. procumbens* extract, Ga-NPs, and Gp-NPs tend to be less stable to NaCl.

![Graph showing pH effect on UV-Vis spectra](image)

**Fig.-6: The pH Effect on UV-Vis Spectra of (A) *G. procumbens*, (B) Ga-NPs, and (C) Gp-NPs. Decreasen in Gallic Acid and Quercetin Release at Alkaline pH is Due to Its Characteristics, which are Unstable at Alkaline pH**

The increasing salt concentration resulted in a hyperchromic shift in the band I (616 nm) of *G. procumbens* extract, Ga-NPs, and Gp-NPs absorption band. Meanwhile, the 0.3 M salt concentration resulted in a hypochromic shift in bands I and II of Ga-NPs and Gp-NPs (Fig.-7).

**The Release of Nano *G. procumbens***

The release of gallic acid and quercetin on Ga-NPs and Gp-NPs at pH 2, 7, and 8.5 is performed for seven hours (Fig.-8). The most significant gallic acid and quercetin release in Ga-NPs and Gp-NPs is at pH 2, where the release percentage was > 2.0%. Meanwhile, the lowest gallic acid and quercetin release in Ga-NPs and Gp-NPs was at pH 7 and 8.5, respectively. In general, gallic acid and quercetin release on Gp-NPs is better than Ga-NPs. It is related to the potential zeta value of both. The Zeta potential value of Gp-NPs is better than Ga-NPs, so the possibility of aggregation of Gp-NPs is lower than Ga-NPs because the stability of Gp-NPs is better than Ga-NPs. Therefore, the ability of Gp-NPS to promote gallic acid and quercetin release Gp-NPs is better than Ga-NPs.

![Graph showing percentage release of gallic acid and quercetin](image)

**Fig.-8: The Percentage Release of (A) Gallic Acid and (B) Quercetin in Ga-NPs and Gp-NPs**
Loading Efficiency (%LE) and Loading Amount (%LA) Nano G. procumbens

Loading efficiency (%LE) expresses the percentage of drug samples successfully trapped in the micelles. The %LE value is calculated by subtracting the total drug sample from the free drug sample that is not entrapped and then divided by the total drug sample. The %LA can be calculated by dividing the number of trapped drug samples by the total mass of nanoparticles. This work calculated the gallic acid compound's %LE and %LA as the major secondary metabolite in nano G. procumbens. Based on the results, the %LE of gallic acid compounds in Ga-NPs and Gp-NPs are 96.236 ± 1.156 and 95.567 ± 0.334%, respectively. These results showed that more than 90% gallic acid of the total gallic acid contained in the G. procumbens extract was encapsulated in each polymer. The %LA values for Ga-NPs and Gp-NPs are 27.496 ± 0.331 and 27.305 ± 0.096%, respectively. Meanwhile, the %LE of quercetin is 99.123 ± 0.155 (Ga-NPs) and 98.585 ± 0.155% (Gp-NPs). The %LA for quercetin compounds from Ga-NPs and Gp-NPs are 28.321 ± 0.044 and 28.167 ± 0.044%, respectively.

Antidengue Activity

Table 2 lists the CC₅₀ and IC₅₀ of extracts of G. procumbens, Ga-NPs, and Gp-NPs. Then, the CC₅₀ values of G. procumbens extract, Ga-NPs, and Gp-NPs are 187.11, >200, and >200 µg/mL, respectively. There was a significant difference between the CC₅₀ values of G. procumbens extract, Ga-NPs, and Gp-NPs. Both nano G. procumbens have a CC₅₀ value higher than G. procumbens extract. This means that the cytotoxicity of Ga-NPs and Gp-NPs is much lower than the G. procumbens extract. Meanwhile, the IC₅₀ values of extracts of G. procumbens, Ga-NPs, and Gp-NPs were 11.22, 7.67, and 12.75 µg/mL, respectively. The extract and both nano G. procumbens actively inhibit the dengue virus. This anti-dengue activity of G. procumbens is in line with the high content of gallic acid. The virucidal effect of gallic acid is correlated with the ability of the compound to inhibit virus attachment to cells and cell-to-cell spread activity partially. Previously, gallic acid contained in P. guajava and the Phyllanthaceae family has successfully addressed dengue viral infection with percent inhibition more significant than 50%. Based on IC₅₀, Ga-NPs are the best candidate for anti-dengue since it has lowest IC₅₀. Although the release of Gp-NPs is better than Ga-NPs, Ga-NPs exhibit the best anti-dengue with the lowest IC₅₀ since it has the highest %LE which means more gallic acid encapsulated by PVA. In other words, there is plenty of gallic acid in Ga-NPs to combat dengue viral infection.

Table 2: Anti-dengue Activity of G. procumbens Extract, Ga-NPs and Gp-NPs

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>CC₅₀ ± SE (µg/mL)</th>
<th>IC₅₀ ± SE (µg/mL)</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>G. procumbens extract</td>
<td>187.11 ± 35.67</td>
<td>11.22 ± 16.68</td>
<td>16.67</td>
</tr>
<tr>
<td>2.</td>
<td>Ga-NPs</td>
<td>&gt;200</td>
<td>7.67 ± 0.78</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3.</td>
<td>Gp-NPs</td>
<td>&gt;200</td>
<td>12.75 ± 1.24</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

Based on the selectivity index (SI), both nano G. procumbens are more selective than the G. procumbens extract. The SI value is the ratio between the toxic concentration of the sample and the activity of the sample’s bioactive components the sample. The SI value 10 is the limit of a sample being said to be a potential drug candidate. The recent results show that G. procumbens extract Ga-NPs, and Gp-NPs can be developed as an anti-dengue treatment. However, nano G. procumbens are more potential because they have higher SI values with low cytotoxicity (CC₅₀) and high anti-dengue activity (IC₅₀) compared to G. procumbens extract (Table 2). The current bioassay result support that nanoencapsulation has the potential to increase bioavailability, increase controlled release, and allow precision targeting of bioactive compounds to increase activity.

CONCLUSION

Gynura procumbens nanoencapsulation has been constructed, namely Ga-NPs and Gp-NPs. The particle size of the Ga-NPs is 161.00 ± 2.17 nm which is more significant than Gp-NPs 136.33 ± 0.47 nm. Both convey stability at temperatures of 30-100°C but are less stable at high pH and the addition of high salt concentrations. Ga-NPs have a higher %LE and anti-dengue activity compared to Gp-NPs. Ga-NPs and Gp-NPs have a high SI index, indicating the ability to increase the anti-dengue activity of G. procumbens.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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