Sustained Release Multiparticulate Oral Drug Delivery System of *Piper betle* Leaf Extract

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Introduction

*Piper betle* Linn. is a climber plant in the family of Piperaceae. It has been extensively used in the traditional herbal remedies in Thailand. The reported pharmacological effects of *P. betle* were antimicrobial\(^8\), anti-oxidant\(^3\), anti-inflammatory\(^7\), anticarcinogenic\(^4,\,^6\), anti-diabetic\(^5\) and xanthine oxidase inhibitory activity\(^2\). The major bioactive constituent in *P. betle* is hydroxychavicol or allylpyrocatechol. Due to the beneficial activities of *P. betle*, the use of the extract of *P. betle* leaf containing the active compound, hydroxychavicol, to prepare as the pharmaceutical dosage forms is interesting. Multiparticulate drug delivery systems such as beads have been used to deliver the active compounds. The active constituent incorporated in the systems could release in a sustained manner. Furthermore, the encapsulated compounds could be protected from the environments; thus, the stability of the encapsulated compounds could be extended. Sodium alginate is a good candidate for preparation of beads due to its excellent properties such as good biocompatibility, good biodegradability and non-toxicity. The encapsulation of the active ingredients is obtained by cross-linking ability of sodium alginate when contact to divalent cations in aqueous solution.\(^1\)

The objectives of this study were therefore to develop sustained release alginate beads containing *P. betle* leaf extract intended for oral delivery of hydroxychavicol and to determine the effects of formulation variables on the physical properties and the *in vitro* hydroxychavicol release.

Methods

**Preparation of *P. betle* extract**

The ground dried leaf of *P. betle* was extracted with 95% ethanol by maceration in the ratio of 1:3 w/v for 2 days. The liquid extract was filtered and concentrated by using a rotary evaporator. The obtained extract of the leaf of *P. betle* was green viscous liquid. The extract was stored in a well-closed container at 2-8 °C till used. The yield of the extract was approximately 14% by weight of the dry leaf. The crude extract of *P. betle* leaf contained 28.40% of hydroxychavicol.

**Quantification of hydroxychavicol by HPLC analysis**

The contents of hydroxychavicol in the crude extract and *in vitro* drug release samples were analyzed by HPLC in an isocratic mode. A C\(_{18}\) column (VertiSep\(^\text{TM}\) UPS C\(_{18}\) column 4.6×250 mm, 5 µm, Ligand Scientific, Bangkok, Thailand) was used as the stationary phase. The mobile phase consisted of 0.1% orthophosphoric acid and acetonitrile in the ratio of 50:50 v/v. The injection volume was 20 µL. The flow rate of the mobile phase was 1 mL/min. The UV detector was set at the wavelength of 280 nm. The retention time of hydroxychavicol was at approximately 7 min.

**Formulation of sustained release alginate beads containing *P. betle* extract**

The beads were prepared by ionotropic gelation method. Sodium alginate was prepared in a concentration of 2, 3 and 4 %w/v in distilled water, and the extract was added into the polymer solution at varying ratio. The mixture was extruded through the syringe with needle No. 26 into calcium chloride solution with constant stirring at 800 rpm. The hardening time after extrusion finished was 20 min. The resultant beads were washed with deionized water. Then, the beads were dried by freeze-drying technique. Beads were then filled into hard gelatin capsules (size 00) to obtained bead amount of 500 mg/capsule. The capsules of beads
were stored in an air-tight glass container at room temperature and protected from light. The bead formulations are shown in Table 1.

**%Bead yield**

Bead yield was calculated using the following equation:

\[
\% \text{Bead yield} = \frac{\text{Amount of dried beads recovered}}{(\text{Amount of drug} + \text{Amount of polymer})} \times 100
\]

**Size of beads**

The diameter of beads was determined on a sample of 50 beads using an optical microscope equipped with a camera.

**%Drug loading and %encapsulation efficiency**

Hydroxychavicol was extracted from the beads by using methanol as a solvent. The beads in methanol were subjected to sonication for 1 h to destroy the bead structure. Then, the obtained mixture was filtered through a Whatman filter paper No. 1. The content of hydroxychavicol in beads was determined by a validated HPLC method. %Drug loading and %encapsulation efficiency were calculated using the following equations:

\[
\% \text{Drug loading} = \frac{(\text{Hydroxychavicol entrapped/Dried beads})}{\times 100}
\]

\[
\% \text{Encapsulation efficiency} = \frac{(\text{Hydroxychavicol entrapped/Theoretical hydroxychavicol content})}{\times 100}
\]

**In vitro drug release studies**

One capsule of each formulation of beads of 500 mg was placed in 500 mL of simulated intestinal fluid pH 6.8 without enzyme. The in vitro drug release was carried out using the USP paddle apparatus. The test was performed at 37 ± 0.5 °C, and the rate of paddle rotation was kept constant at 75 rpm. The formulations were subjected to the release studies for 6 h. Samples were withdrawn and replaced with fresh medium at 5, 10, 30, 60, 120, 180, 240, 300 and 360 min. The concentrations of hydroxychavicol were assayed by a validated HPLC method. The test was repeated in triplicate for each formulation. The data was reported as mean ± SD. A plot of the cumulative %hydroxychavicol released against time was constructed to illustrate the drug release profiles.

**Results**

**Development of sustained release beads of *P. betle* leaf extract**

The beads were prepared by ionotropic gelation method via a complexation between alginate and divalent cations; Ca^{2+}. The resultant beads of all formulations exhibited a spherical in shape and dark green in color. The yield of the obtained beads of all formulations was in the range of 30 to 35%. There was no difference of the yield among all formulations. The bead diameter was similar in all formulations in the range of 1.27 to 1.68 mm. However, the beads containing higher amount of sodium alginate tended to show larger particle size. Higher amount of polymer yielded higher viscosity of the mixture when extruded through the syringe during bead preparation; thus, the droplets of the polymer mixture were large, and this cause larger bead size when cross-linking with calcium ions occurred.

**Table 1** The compositions of sustained release alginate bead formulations, %drug loading and %encapsulation efficiency

<table>
<thead>
<tr>
<th>No.</th>
<th>Sodium alginate (%w/v)</th>
<th>CaCl_2 (%w/v)</th>
<th>Extract:Alginate ratio (w/w)</th>
<th>Drug loading (%)</th>
<th>Encapsulation efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>2</td>
<td>2.5</td>
<td>1:3</td>
<td>30.80 ± 2.11</td>
<td>80.94 ± 1.03</td>
</tr>
<tr>
<td>F2</td>
<td>3</td>
<td>2.5</td>
<td>1:3</td>
<td>23.90 ± 1.37</td>
<td>72.68 ± 1.62</td>
</tr>
<tr>
<td>F3</td>
<td>4</td>
<td>2.5</td>
<td>1:3</td>
<td>19.53 ± 2.14</td>
<td>63.53 ± 2.10</td>
</tr>
<tr>
<td>F4</td>
<td>3</td>
<td>2.5</td>
<td>1:1</td>
<td>18.41 ± 1.56</td>
<td>57.84 ± 2.30</td>
</tr>
<tr>
<td>F5</td>
<td>3</td>
<td>2.5</td>
<td>1:2</td>
<td>21.78 ± 2.43</td>
<td>59.73 ± 2.54</td>
</tr>
<tr>
<td>F6</td>
<td>3</td>
<td>5</td>
<td>1:3</td>
<td>29.45 ± 2.03</td>
<td>77.52 ± 2.58</td>
</tr>
<tr>
<td>F7</td>
<td>3</td>
<td>10</td>
<td>1:3</td>
<td>35.81 ± 1.16</td>
<td>81.61 ± 1.41</td>
</tr>
</tbody>
</table>

**%Drug loading and %Encapsulation efficiency**

The results of %drug loading and %encapsulation efficiency are shown in Table 1. Drug loading and encapsulation efficiency were decreased with increasing the concentrations of sodium alginate polymer, but higher calcium chloride concentrations improve the drug loading and encapsulation efficiency which acted as a cross-linking agent. The ratio of the extract to sodium alginate of 1:3 yielded higher drug loading and encapsulation efficiency than the ratio of 1:2 and 1:1, respectively.
**In vitro drug release**

The in vitro drug release showed that the release rate and extent of hydroxychavicol were influenced by the concentrations of polymer, calcium chloride and the ratios of the extract and polymer. As shown in Figure 1, greater concentrations of sodium alginate resulted in the slower release of hydroxychavicol. Higher calcium chloride concentrations also retarded the release of the encapsulated drug (Figure 2). The ratio of the extract and sodium alginate did not significantly influence the drug release profiles although the beads containing extract to sodium alginate in the ratio of 1:1 (F4) exhibited slightly higher release amount of hydroxychavicol at the first hour compared to F5 and F2 beads, respectively (Figure 3).

![Figure 1](image1.png) **Figure 1** Release profiles of hydroxychavicol from the alginate beads containing *P. betle* extract with different amounts of sodium alginate in simulated intestinal fluid pH 6.8. Data represents the mean ± SD (n = 3)

![Figure 2](image2.png) **Figure 2** Release profiles of hydroxychavicol from the alginate beads containing *P. betle* extract with different concentrations of calcium chloride in simulated intestinal fluid pH 6.8. Data represents the mean ± SD (n = 3)

![Figure 3](image3.png) **Figure 3** Release profiles of hydroxychavicol from the alginate beads containing *P. betle* extract with different ratios of *P. betle* extract to sodium alginate in simulated intestinal fluid pH 6.8. Data represents the mean ± SD (n = 3)
The optimum formulation of sustained release alginate beads of *P. betle* extract

According to the drug loading, encapsulation efficiency and the drug release, F1 bead formulation was selected as an optimum formulation. F1 beads showed high drug loading and encapsulation efficiency. The release of hydroxychavicol was found to be a sustained release profile with the highest total released extent.

Discussion

Hydroxychavicol, the active compound in *P. betle* extract has been shown to exhibit various types of pharmacological properties. In this study, the *P. betle* leaf extract was encapsulated in the multiparticulate oral drug delivery systems called beads which were manufactured by ionotropic gelation between sodium alginate and calcium chloride. The encapsulated *P. betle* extract was successfully prepared with a sustained release profile of hydroxychavicol. Different pharmaceutical factors influencing the loading, the encapsulation efficiency and the release of hydroxychavicol were determined.

Increasing the sodium alginate concentrations provided higher viscosity of the mixture that might possess more space of the polymer resulting in smaller area for the active drug to be encapsulated in the beads. This led to lower drug loading and encapsulation efficiency. Increasing the ratios of extract to sodium alginate resulted in higher loading and encapsulation efficiency of hydroxychavicol. This might be due to the fact that the lower amount of the polymer was not adequate for the encapsulation of the entire amount of the extract especially when the extract amount increased. Higher concentrations of calcium chloride afforded higher ionotropic complexation between sodium alginate and calcium ions. This provided denser wall of the beads; therefore, the active compound, hydroxychavicol was rapidly entrapped within the bead structure. Thus, it was found that the hydroxychavicol content and encapsulation efficiency were improved by increasing calcium.

Higher concentrations of sodium alginate led to a stronger network of the bead structure that was a major barrier for the permeation of aqueous medium into the beads and the consequent drug release. This resulted in a slower release rate of the incorporated drug. Higher concentration of cross-linking agent caused denser structure of the beads and then the release of the drug was retarded. The finding was in accordance with other previous report.

Conclusion

The alginate beads containing *P. betle* extract showed a sustained release of hydroxychavicol. The study showed that the concentrations of alginate, the amounts of calcium chloride and the ratios of the extract to alginate affected the loading and encapsulation efficiency of hydroxychavicol in the beads. Moreover, these variables also influenced the hydroxychavicol release. The developed multiparticulate oral sustained release formulation of *P. betle* extract could be potential to be used as the alternative nutraceuticals.

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References