NANOENCAPSULATION OF GRAVIOLA (Annona muricata L.) SEED BY PRODUCTS ETHANOLIC EXTRACT FOR BREAST CANCER ACTIVITY

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Abstract

Breast cancer is the most second prevalent cancer worldwide and causes high numbers of deaths among woman every year. According to the World Health Organization (WHO), approximately 8-9% woman worldwide have an affected breast cancer. Annona muricata L. commonly known as Graviola has demonstrated that the seed use of natural products as anticancer agents in breast cancer. The bioactive compound such as flavonoids in Graviola reported exhibiting potent and uncommon selective growth inhibition against of breast cancer. This research to a known formula of nanoencapsulation of ethanolic seed extract of Annona muricata L. Using phytochemical screening, nanoencapsulation method, and Particle Size Analyzer (PSA). Phytochemical screening was conducted using qualitative methods to assign the relative abundance of the different phytochemicals. The nanoencapsulation process was using thin layer method to improve functionality as anticancer. The nanoencapsulation size and stabilities of Graviola seed extract are measured by Particle Size Analyzer. Phytochemical screening of the extract revealed that the seed has secondary class metabolite compounds such as alkaloids, flavonoids, phenolics, and saponins. The nanoencapsulation process was made using ethanolic extract and maltodextrin with formula 1:1, 1:2, 1:3, and 1:4. The result showed nanoencapsulation with formula 1:1 had Z-average 11,1 nm; Pi value 0,187; and the stability of nanoencapsulation product have shown by zeta potential -44,4 mV. The data showed that seed extract of Graviola has beneficial properties in alternative medicine for breast cancer.

Keyword: Breast Cancer, Graviola Seed, Nanoencapsulation

INTRODUCTION

Breast cancer is the most second prevalent cancer worldwide and causes high numbers of deaths among woman every year. The rates of mortality this disease have increased in the developed countries. The therapy for breast cancer has developed with natural products, because the properties of it not harming healthy cells.

Natural product has been used as traditional medicines to satisfy their primary health care needs. Many cancer patients use herbal medicines as alternative medicines including phytochemical compounds (Eisenberg et al., 1998). Several phytochemical compounds from natural products capable of anticancer agents such as flavonoid. Bioactive compounds such as alkaloids, flavonoids, tannins, and phenols were considered to be most important. The phytochemical research that has been done based on the ethnopharmacological information forms the effective approach in the discovery of new anti-infective agents from higher plants (Duraipandiyan et al., 2006).

Annona muricata L. commonly known as Graviola, belongs to the Annonaceae family, its native in Central America and widely distributed in Asia countries (Alassane et al., 2004). The bioactive compound in the seed of Graviola has demonstrated to be selectively toxic against on cancer cell without harming the healthy cell. Flavonoid was reported to exhibit cytotoxic activities against breast cancer cell line.

Encapsulation with the polymer has been largely applied to protect sensitive substance from adverse effects of the surrounding environment such as exposure to oxygen, heat, and light can cause loss or reduction bioactivities. The nanoencapsulation method may be used to preserve active compounds. Furthermore, this method can improve aqueous solubility, control and target the delivery of active compounds (Weiss et al., 2006).
Considering the potential bioactivity of the Graviola fruit by-product extract and their potential as anticancer agents, the aim of this study was to produce nanoencapsulation of seed Graviola byproduct extract using the thin layer method was made by extract ethanolic seed of Graviola and maltodextrin to cover bioactive compounds in the seed of graviola.

**METHODS**

**Preparation of extracts**

The seeds of graviola were collected at merchant juice in Sleman, District of capital city of Yogyakarta. The seeds were washed with water and dried below on sunlight, then grounded using a blender. The obtained powder was soxhlet with ethanol as solvent, the suspension was filtered through filtered paper and filtrate was evaporated to dryness at 80°C, using a rotary evaporator. The obtained extracts were stored and used for further analysis.

**Screening phytochemical**

Determination of flavonoids, alkaloids, saponins, phenols

Phytochemical screening was conducted using qualitative methods to assign the relative abundance of the different phytochemicals. Flavonoids, alkaloids, saponins, and phenols content was determined using different reagent. The extracts placed in the test tube and added reagent respectively. Aluminium chloride (AlCl₃) was used for determined flavonoids. Drugendorff reagent was used for determined alkaloids content. FeCl₃ was using to determined phenols content. Aquadest added to the extract for determined saponins

**Nanoencapsulation**

The nanoencapsulation process was made by seed extract and maltodextrin with formula 1:1; 1:2; 1:3; 1:4, used thin layer drying method. The extracts were made into the emulsion by using tween 80. The emulsion of the extracts was mixed with maltodextrin solution. The obtained compound was used for the next steps. Nano process was made by using ultra turax mixer at 600 rpm for 10 minutes, this process will slice the size of extracts into a small piece. The obtained solution will analysis by PSA to know particle size, distribution of particle, and stability of seed extracts.

**RESULT**

**Pytochemical content of A. muricata**

The result of this study showed that extract has bioactive compounds such as flavonoids, alkaloids, phenols, and saponins (table.1) (Figure.1). The yield of screening phytochemicals revealed that the seed extracts rich in secondary class metabolite compounds. Flavonoid is bioactive compounds that have potential as anticancer agents for therapy of breast cancer.

<table>
<thead>
<tr>
<th>Type of screening</th>
<th>Yield</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Orange</td>
<td>(+) alkaloids</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Sorrel</td>
<td>(+) flavonoids</td>
</tr>
</tbody>
</table>

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87
The result showed the encapsulation process of Graviola seed extracts has succeeded into nano size (table.2), it showed by the grand average of particle size. Nanoparticle is particle between 1 and 100 nanometers (nm) in size with a surrounding interfacial layer. The data nanoencapsulation with formula 1:1 have Z-average 11.1 nm; Pi value 0.187, it means that seed extracts have same particle size or have monodispersion property. The stability of nanoencapsulation product have shown by zeta potential. It is Surface charge of particle in turn indicates how stable is particle in the suspension system. Particle with higher zeta potential become unstable, due to it have higher surface energy, higher the surface charge will make higher repulsion between particles. A data showed zeta potential -44.4 mV for formula 1:1 more stable than others.

**CONCLUSION**

Overall, the existing knowledge about graviola extract agent have been carried out based on qualitative method with screening phytochemical. The extract of A. muricata has flavonoids...
compound that can be potent as anticancer agents, and nanoencapsulation formula 1:1 have good properties as anticancer agents for therapy breast cancer. However, further studies are required to verify the exact flavonoid properties of the extract using LCMS-MS (Liquid chromatography-mass spectrometry) or FTIR (Fourier Transform Infrared) and verify the mechanisms action as anticancer. Even though, several studies have shown positive action of graviola as anticancer, clinical trials to test the extract are required to reinforced validity data.

REFERENCE