Cytotoxicity and antiproliferative activity of ethanol and ethyl acetate fractions from polymeric nanoparticles of green tea leaves (Camellia sinensis) in breast cancer cell line MDA-MB-132

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ORIGINAL ARTICLE

Cytotoxicity and antiproliferative activity of ethanol and ethyl acetate fractions from polymeric nanoparticles of green tea leaves (*Camellia sinensis*) in breast cancer cell line MDA-MB-132

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ABSTRACT

Green tea (*Camellia sinensis*) has benefits. Its main potential content is epigallocatechin gallate, which has many bioactivity and pharmacological properties. However, herbal medicines have limitations on low solubility and stability. A nanoparticle delivery system is a perfect form of active ingredient development, because it can mediate the increase in solubility, dissolution rate, and strength of a targeted delivery system. This study aimed to make and test the formulation of the ethanol and ethyl acetate fraction from green tea leaves in the form of a nanoparticle delivery system using chitosan biopolymer as the primary carrier polymer combined with sodium tripolyphosphate as a crosslinker and then carried out the tests on the MDA-MB-231 breast cancer cell line. The results showed that the particle size value was 199.7 nm, the zeta potential was-56.7 mV, and the polydispersity index was 0.337. X-ray diffraction and differential scanning calorimetry test results showed that the *C. sinensis* fraction was perfectly dispersed molecularly in the nanoparticle system. The results of the cytotoxic test on the MDA-MB-231 breast cancer cell line obtained IC50 values for both fractions, namely 10.70 μg/mL (nano ethanol fraction) and 12.72 μg/mL (nano ethyl acetate fraction). This result showed a significant increase in anticancer activity in both fractions compared to those not formulated ($P < 0.05$). These results also show that the *C. sinensis* tea fraction formulated in a nanoparticle delivery system has a great potential as a new therapeutic agent for breast cancer.

Key words: Cytotoxicity test, drug delivery system, ethanol, ethyl acetate fraction of *Camellia sinensis* green tea leaves, MDA-MB-231 breast cancer cells, nanoparticle technology

INTRODUCTION

The advantage of herbal drugs has increased because they have fewer side impacts than engineered drugs. The obstacle in herbal products is the active substances in herbal

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medicinal preparations, which are difficult to penetrate the lipid membranes of the body's cells.[1,2]

Nanoparticles are a technology aiming to modulate the size and dosage form in 10–1000 nm.[3] Moreover, nanotechnology can modify surface characteristics and particle size, so that herbal medicines target a particular receptor or part of an organ. In addition, the release of active compounds can be controlled to minimize the side effects of herbal medicines.[3,4]

Cancer is a disease caused by abnormal cells in body tissues that develop rapidly and uncontrollably.[5] The recapitulation of cancer early detection results in Indonesia from 2006 to 2015 show that the number of sufferers is 1.9 million.[6] Breast cancer is one of the most feared types of cancer, especially for women. Breast cancer is the growth of abnormal cells proliferative in breast tissue.[7] Cancer treatment, in this way, has several drawbacks. Surgery is generally ineffective for cells that have undergone metastases, while chemotherapy has not given optimal results, because the action of drugs is not specific and is relatively expensive.[1,8]

Based on this, the use of herbs as an alternative cancer treatment is increasing. The raw materials for traditional medicines are easy to obtain, safe, relatively inexpensive, and have a fewer side effects than synthetic drugs.[9,10] One of the traditional plants with anticancer properties is green tea. Green tea contains various chemical components, including catechins. The catechin compound in tea is epigallocatechin-3-gallate (EGCG). EGCG has been shown to inhibit cancer cell growth and plays an essential role in stimulating apoptosis or programmed cell death.[5]

Thus, it is necessary to formulate green tea leaves (*Camellia sinensis*) and test their cytotoxicity and proliferative activity on the MDA-MB-132 breast cancer cell line.

MATERIALS AND METHODS

Materials

Green tea leaves (*C. sinensis*) deionized water, CH3COOH, C2H5OH, C4H8O2, KBr, chitosan, Na5P3O10, carrageenan, PBS (phosphate buffer saline), breast cancer cells in the MDA-MB-231 cell line, RPMI 1640 Gibco penicillin-streptomycin, fetal bovine serum DMSO, RPMI, MTT, SDS, and aluminum foil.

Research methods

This type of research is an experimental research (laboratory scale) with a randomized posttest-only control group design. Five main steps were carried out in this research. Then proceeded with the manufacture of nanoparticles using a spray pyrolysis tool, the nanoparticle characterization process, and in the final stage, *in vitro* testing of the cytotoxicity activity of the test preparation nanoparticles against the MDA-MB-231 breast cancer cell line.[7,11]

RESULTS

Molecular interaction results between chitosan compound and sodium tripolyphosphate as polymeric nanoparticle careers.

The bond distance (interaction between chitosan and sodium tripolyphosphate) value is 2.22 A, and the bond energy is −0.081 kcal/mol.

The molecular ratio is 1:1. An illustration of the molecular interaction between chitosan compounds and sodium tripolyphosphate is shown in Figure 1.

Measurement of particles, polydispersity index, and zeta potential of ethanol fraction polymeric nanoparticles of green tea leaves (*C. sinensis*).

From the test results, the particle measurements in each formulation (F1, F2, and F3) were 270.1, 247.7, and 199.7 nm, respectively. Formulation F3 has the smallest particle size, because the ratio of chitosan and sodium tripolyphosphate is in the equimolar equilibrium of 4:1, as shown in Table 1 and Figure 2.

Table 1: Measurement of nanoparticles from green tea leaves (*Camellia sinensis*)

Parameter	Formula		
	F1	F2	F3
Particle size (nm)	270.1	247.7	199.7
Polydispersity	0.602	0.380	0.337
Index zeta potential (mV)	−37.2	−50.5	−56.4

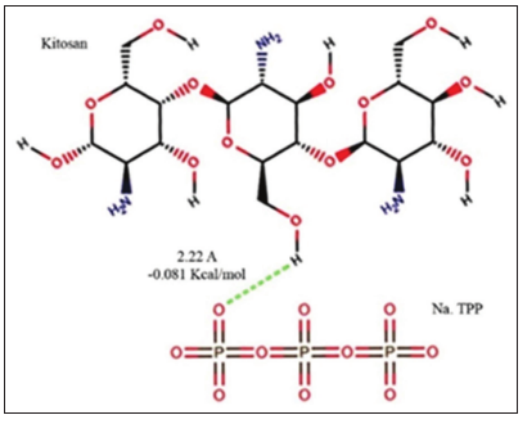

Figure 1: Molecular interaction of chitosan and sodium tripolyphosphate

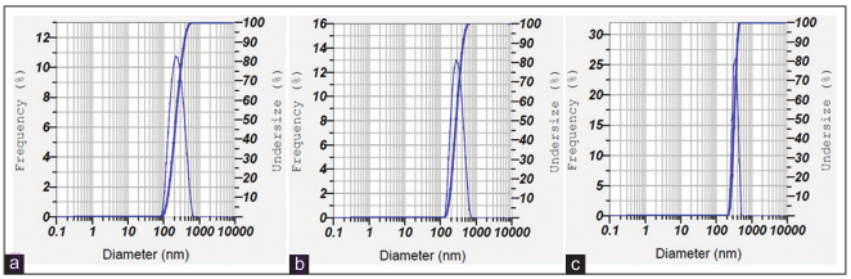

Figure 2: Particle size distribution on the three formulations, namely: (a). F1, (b). F2, and (c). F3

From this test, the zeta potential value is also obtained, which is the value that becomes the stability parameter of the dispersion system. Furthermore, the F3 formulation had a higher zeta potential value than F2 and F1.

X-ray diffraction (XRD) characterization on ethanol fraction polymeric nanoparticles of green tea leaves.

The diffractogram pattern shows the results of 2Θ angle and intensity values. It depends on the strength of the intermolecular electron bonds in the molecule. It is shown in Figure 3.

Characterization of differential scanning calorimetry (DSC) on ethanol fraction polymeric nanoparticles of green tea leaves (*C. sinensis*).

In the DSC curve, sodium tripolyphosphate shows the peak of the endothermic phase at a temperature of 120.06°C, which is indicated as the material's melting point. This pattern also illustrates that sodium tripolyphosphate is in a crystalline form. Meanwhile, chitosan and carrageenan polymers demonstrated a glass-transition pattern, which indicated an amorphous form.

Cytotoxicity test results of green tea leaf (*C. sinensis*) on breast cancer cell line.

The results of the two fractions formulated in the form of nanoparticles could significantly increase breast cancer's effectiveness. The test data for the ethanol fraction have an IC50 value of 10.70 µg/mL and the ethyl acetate fraction have an IC50 value of 12.72 µg/mL.

DISCUSSION

The interaction between chitosan and Na5P3O10 (the bond distance) value is 2.22 A, the bond energy is − 0.081 kcal/mol, and the molecular ratio is 1:1. This result is also the basis for choosing the mass balance formula for chitosan molecules and Na5P3O10 molecules. The ratio of 1:1 mole for the chitosan and Na5P3O10 has an equivalent mass ratio of 4:1 in the optimization results of the best formula.[12,13]

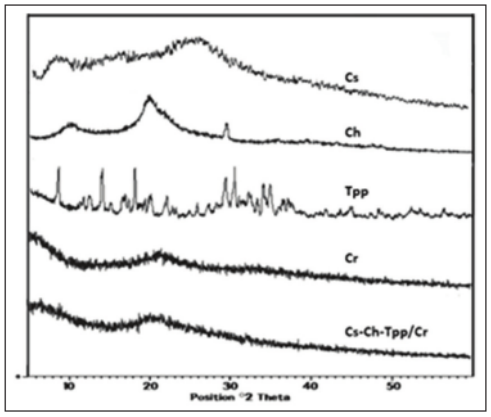

Figure 3: X-ray diffraction characterization results

The smallest particle size is found in the F3. The proper ratio will make chitosan and Na5P3O10 completely react in the ionic gelation process and create a perfect form and stable nanoparticle system. In contrast, the F2 and F1 ratios have a more significant ratio. The result of this comparison will be the presence of free chitosan molecules that do not completely react with Na5P3O10. Chitosan in this free state has absorption capability, and selectivity.[14] The polydispersity index value describes the uniformity of particle size of the nanoparticle system. The small polydispersity index value represents that the nanoparticle system obtained is monodisperse. Moreover, the large polydispersity index value describes the polydispersity system.

The result shows that the lower the chitosan-to-Na5P3O10 ratio, the lower the polydispersity index.[15] Then, the potential zeta value test was carried out to represent the accumulation of charges that form an electrical double layer on the surface of each nanoparticle. It shows that the higher the potential zeta value, the better stability. In Table 1, F3 has the biggest zeta potential value; as a result, the chitosan-to-Na5P3O10 ratio significantly affects the value of particle size ($P < 0.05$), polydispersity index, and zeta potential. Finally, F3 has the best characteristics.[16]

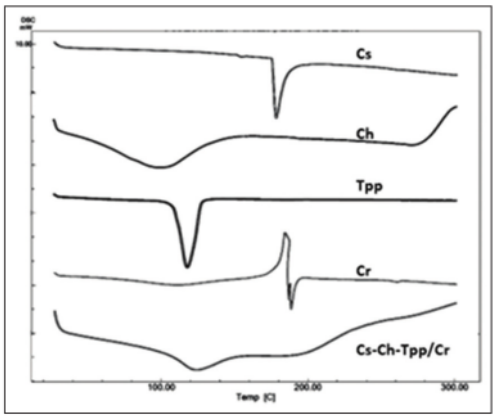

Figure 4: Differential scanning calorimetry results

The test samples *C. sinensis* and chitosan both showed a semicrystalline pattern in the XRD analysis, with diffractogram peaks at angles of 2Θ 25–27o (*C. sinensis*) and 19.72o (chitosan) [Figure 3]. The diffractogram data for *C. sinensis*-chitosan-tripolyphosphate-carrageenan nanoparticles showed an amorphous pattern. The crystalline lattice of *C. sinensis* is no longer visible due to this formula. It indicates that *C. sinensis* has been molecularly distributed in the nanoparticle matrix. Furthermore, chitosan is a semicrystalline polysaccharide.[17,18]

According to the DSC analysis, chitosan compounds peak at roughly 100°C [Figure 4]. The evaporation phase of H2O bound in the chitosan and alginate molecules causes this response. This formula does not reveal the sodium tripolyphosphate crystalline component's endothermic or exothermic peaks. These results correlate with the XRD results in the previous test, proving that the Cs-Ch-Tpp/Cr NPs formula molecularly has dispersed *C. sinensis* in the nanoparticle matrix system and encapsulated in the nanoreservoir system.[19]

The results of cytotoxicity tests [Figure 5] reveal that the nanoparticle dosage has a higher bioavailability. This result showed a significant increase in anticancer activity in both fractions compared to those not formulated ($P < 0.05$). Furthermore, using the chitosan biopolymer increases drug penetration across cancer cell membranes.[20] This is due to the chitosan polymer's cationic charge, which strongly favors negatively charged cell membranes.[21]

These impressive results also cannot be separated from the role of carrageenan biopolymers. Carrageenan is known to influence the growth cycle of cancer cells, as in previous studies which showed that carrageenan could inhibit the migration of breast cancer cells MDA-MB-231.[11] The mechanism of action of carrageenan in inhibiting the growth of cancer cells has also been investigated, capable of

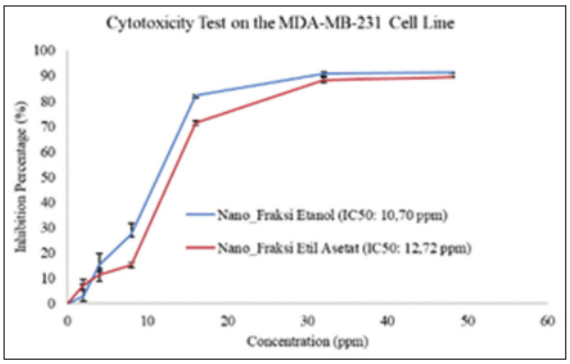

Figure 5: Cytotoxicity test results

inducing the process of apoptosis.[7] This caused an increase in the cytotoxicity of the ethanol fraction and the ethyl acetate fraction of *C. sinensis* green tea leaves encapsulated with carrageenan polymer against the MDA-MB-231 breast cancer cell line.

CONCLUSION

F3 had the best physicochemical characteristics of the other formulas based on the results. XRD and DSC test results have also shown that the ethanol fraction of *C. sinensis* is perfectly dispersed molecularly in the nanoparticle system. The results of the cytotoxic test on the MDA-MB-231 breast cancer cell line showed that nanoparticles could increase the effectiveness of anticancer breast cancer.

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Conflicts of interest

There are no conflicts of interest.

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