

The Potential of *Camellia sinensis* Extract to Prevent HIV/AIDS Transmission Risk Through the Inhibition of Syncytium, GP120, and GP41 Formation

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The Potential of *Camellia sinensis* Extract to Prevent HIV/AIDS Transmission Risk Through the Inhibition of Syncytium, GP120, and GP41 Formation

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Abstract

Until now, HIV/AIDS is still a public health issue that needs serious attention. Vaccines for HIV currently fare far from expectations. Thus, the development of an effective, safe, and affordable anti-HIV treatment is essential to saving the lives of individuals already infected and at-risk with HIV. One of the natural ingredients that is thought to have a potential as an antiviral is green tea (*Camellia sinensis*). The largest green tea contents are flavonoids and epigallocatechin gallate (EGCG) that are suspected to have anti-HIV-1 effects by preventing the binding of gp120 and gp41 to T cell CD4 molecules. This study aims to analyze the potential of green tea extract in inhibiting the activity of HIV-1 infection through inhibitions to the formation of Syncytium, gp120, and gp41 and its development as a preclinical therapy based on the content of bioactive compounds contained in green tea extract. The study proves that green tea extract 10mg/ml has the ability to inhibit the formation of Syncytium and inhibit the attachment of gp120 and gp41 HIV virus to CD4 T cells in vitro. Thus, the process of HIV infection and fusion to target cells can be inhibited so that HIV/AIDS transmission can be inhibited. The conclusion is that green tea extracts, containing EGCG components, may be used as herbal-based anti-HIV candidates.

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Introduction

HIV/AIDS is still a national and global problem that needs serious attention. The presence of HIV/AIDS infection affects many health, medical, social, economic, and cultural issues.¹ Currently, Indonesia is ranked as the fastest country in HIV transmission in Asia.² This is evident from the number of reported cases of HIV/AIDS every year that has been increasing significantly, especially in young and productive age groups. If these conditions remain left without serious mitigation, then HIV-related morbidity and mortality from AIDS will keep increasing.

Until the present day, HIV/AIDS is still a

public health problem that needs serious attention like an unbreakable iceberg. This is evident from the number of reported cases of HIV/AIDS every year that keeps increasing significantly. According to the AIDS Prevention Commission, the data from the Ministry of Health of the Republic of Indonesia up to March 2009 showed that the number of sufferers reached 16,964 people. Cases of HIV/AIDS are growing and have started to become worrying. In Indonesia today, it is found that the largest proportion is in the age group of 20-29 year olds, making up as much as 53% of the cases. The main problem facing people with HIV/AIDS in the world is because the HIV vaccine to date is still far from expectations. Thus, the development of an effective, safe, and affordable anti-HIV treatment is essential to save the lives of individuals already infected and at-risk with HIV.

The immunodeficiency condition that accompanies HIV/AIDS sufferers results in the interruption of the production of cytokines, among them IL-1 and TNF- α , which impairs the function of phagocytosis Polymorphonuclear (PMN) and macrophages.³ T-cell CD4 is a

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glycoprotein on a T-cell surface that plays an important role in the introduction of antigens by T-cells. CD4 also acts as a receptor for HIV-1 because the viral cell wall that is glycoprotein (gp) 120 binds to CD4 in domain D1, and the interaction among them will infect CD4 T-cells.⁴ Although intensive HIV/AIDS treatment efforts have been undertaken, until now a drug has not been found that can overcome the spread of the HIV virus in a satisfactory manner. This is because HIV can avoid the body's defense mechanism caused by antiviral drugs. Therefore, it would be interesting to find a molecule that would block the gp120+ binding to CD4 in an effort to decrease HIV activity.

Until now, with the high price of medicines, the Ministry of Health recommends a *Back to nature* approach, which is a proper recommendation. This is because the material is easy to obtain at a cheap price so it is affordable for all levels of society and can be manufactured by everyone. The natural ingredient that is suspected to have antiviral effects is green tea, consisting of several active ingredients from *Epicatechin* (EC), *Epigallocatechin* (EGC), *Epicatechin gallate* (ECG), to *Epigallocatechin gallate* (EGCG). Components other than *Catechin* are *Theaflavin* and *Cofein*.^{5,6}

It was reported that *Catechin* acts as an antioxidant, anticancer, antifungal, and antiviral substance.⁷⁻⁹ Although we examined the effects of EGCG on some types of viruses and EGCG showed great benefits, the detailed mechanisms of antiviral activity from EGCG are unclear.

Some researchers emphasize that polyphenolic fraction, especially EGCG (*Epigallocatechin gallate*) in *green tea* is the largest component that has the activity to inhibit HIV-1 infection in human CD4 T-cells¹⁰, while *macrophages* on *Dose-dependent manner* protects the attachment of HIV gp120 against the CD4 molecule and deactivates the virion. This inhibition can be achieved at a physiological concentration of 6 mmol/L,¹¹ so EGCG significantly inhibits the production of HIV antigen p24. Thus, the anti-HIV EGCG natural ingredient is an alternative candidate in HIV treatment. EGCG, which is reported to inhibit HIV replication through several stages of *reverse transcriptase* and *protease enzyme* activities blocking gp120 interactions with CD4.¹²

Theaflavin derivatives and catechins are the main group of polyphenols in green tea.

Some tea polyphenols, especially galloyl moiety, can inhibit HIV replication by various mechanisms. *Theaflavin* is a potent natural antioxidant.¹³ Polyphenols can inhibit the entry of HIV virus into target cells by blocking HIV *Envelope glycoprotein-mediated membrane fusion*. The inhibiting activities of polyphenol correlates with its ability to block gp41 formation.¹² Based on *computer-aided molecular docking analyses*, a tea polyphenol named *Theaflavin-3,3'-digallate* (TF3) will bind to the *highly conserved hydrophobic area* on the *Central trimeric surface* by *N-terminal heptad repeats of gp41*.¹³

This complete mitigation will minimize HIV morbidity and mortality from AIDS while cutting transmission of the HIV infection. Until now, most of the FDA-approved anti-HIV drugs are *reverse transcriptase inhibitors* (RTIs).⁶ Although intensive HIV/AIDS treatment efforts have been undertaken, until now a drug has not been found that can overcome the spread of HIV virus in a satisfactory manner. The purpose of this study is to produce an anti-HIV phytopharmaca drug from an indigenous herb from Indonesia, namely green tea (*Camellia sinensis*).

Based on this, green tea is the source of anti-HIV agents. Thus, the natural ingredients of green tea extract with the active compound content can be used as anti-HIV-1 and is an alternative candidate in HIV/AIDS treatment. Nevertheless, the production of a herbal medicine product necessitates clinical trials. Remedies made from ingredients native to Indonesia need to be investigated for potential obstacles to the HIV virus in order to obtain potential effects, taking into account the humanitarian factors in which these drugs must be realized because until now there has been no potent anti-HIV drugs, let alone one that comes from Indonesia. Thus, it can be expected that this medicine will improve the quality of life of people living with HIV and prolong life expectancy. Based on the above information, various strategic steps are needed to tackle HIV infection through research.

Materials and Methods

The unit of research analysis is PBMC culture of CD4 T cells of people with HIV/AIDS. The PBMC CD4 T cells 5×10^6 Cells / ml culture was induced by TCID 501 x 103/ml virus. After

Giant cell (syncytic cell with indirect immunostaining test), the enlarged PMBC cells form giant cells after infection with HIV, the culture was given treatment of green tea extract 10 mg/ml and checked for the presence of syncytium cells. For the examination of gp120 and gp41, the cells were attached to a coverslip using polylysine D. After that, the culture was fixated by using Paraformaldehyde 3.7%. Cells were then saturated with 2% BSA/PBS. The cells were then incubated for 1 hour with a primary antibody (gp41 and gp120) of 1/100 dilution in room temperature, washed three times with PBS, then incubated for 1 hour more with a secondary antibody conjugated with FITC 1/100 dilution. A coverslip was then pasted on a preparatory glass with a medium mounting. To obtain an accurate result, the indirect Immunostaining results were analyzed by a convolution microscope to see the expression of gp120 and gp41. The examination of gp120 and gp41 levels was performed by the ELISA immunoassays method.

Results

The activity of *Camellia sinensis* in gp120 and gp41 (Figure 1).

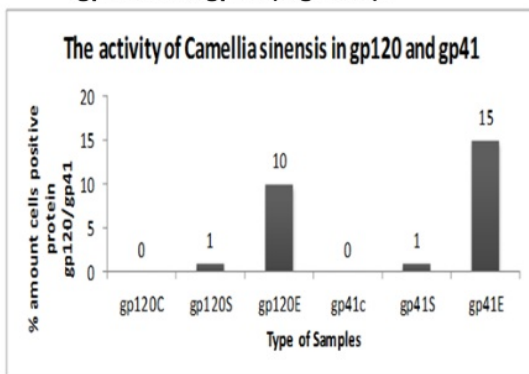
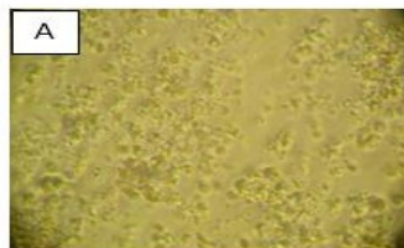


Figure 1. The Green Tea Extract Ability in Binding gp120 and gp41.

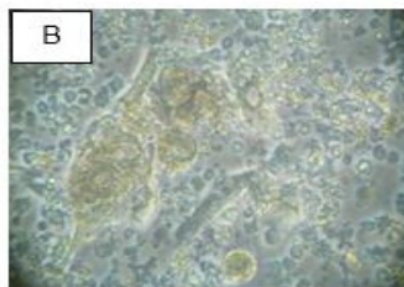
Notes:

- Gp120 C: PBMC CD4 culture + TCells
 - Gp120 S: PBMC CD4 culture + HIV-induced T-cells
 - Gp120 E: PBMC CD4 culture + HIV-induced T-cells and green tea extract 10 mg/ml
 - Gp41 C: PBMC CD4 culture + T-Cell
- Giant cell Cell Screening Results with Indirect Immunostaining (Figure 2).**

Control without



PBMC + Virus



PBMC + Virus extract

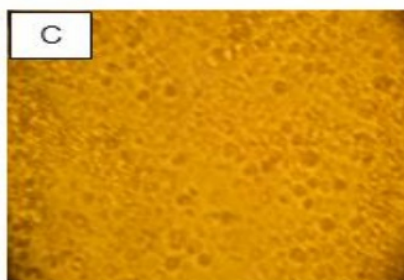


Figure 2. Results of PBMC Culture of HIV/AIDS Sufferers.

Notes:

- A. PBMC CD4 culture + T Cells.
 - B. PBMC CD4 culture + virus-induced T Cells.
 - C. PBMC CD4 culture + T Cells containing virus and given green tea extract 10mg / ml.
- In virus-induced PBMC cultures, syncytium cells formed. After the administration of green tea extract 10mg/ml, the Syncytium did not form.
- gp41 S: PBMC CD4 culture + HIV-induced T-cells.
 - gp41 E: PBMC CD4 culture + HIV-induced T-cells and green tea extract 10 mg/ml.

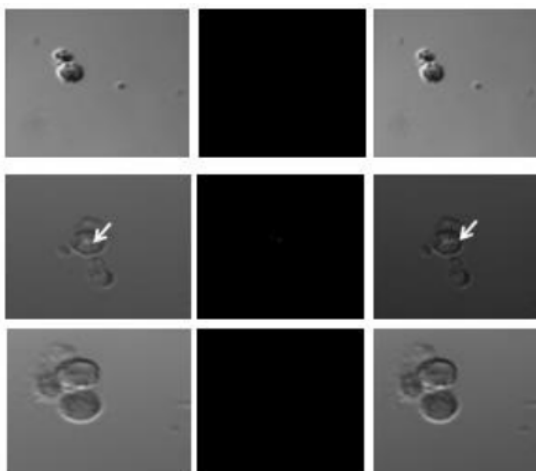


Figure 3. Results of Examination of gp41 and gp120.

Discussion

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The course of HIV infection into the body is initiated by a 120 gp receptor bond on T cell lymphocyte CD4. However, the bond will be stronger with the help of a gp41 receptor. With the mediation of this gp41, the virus will fuse with the target cell membrane.¹⁴ Medicinal plants have a key role in the modern development to study the biological activity of the substances of the bioactive materials they contain. Traditional medicine through the use of medicinal plants can be harmonized and used for the development of disease therapy. Some researchers¹⁵ emphasize that polyphenolic fraction, especially EGCG (*Epigallocatechin gallate*) in *green tea* is the largest component that has the activity to inhibit HIV-1 infection in human T-cells' CD4, while macrophages on *Dose-dependent manner* protects against the attachment of HIV gp120 to the CD4 molecule and deactivates the virion.

The researchers argue that green tea can be used as an *agent* of the HIV antivirus. The bond between HIV and *Human* T cells is the first step in the process of HIV infection. The CD4 molecules serve as a target of the bond between HIV *vesicles* and play a role in the infection process. The high ability of green tea extract in binding gp41 causes the *viral* ability to fuse on inhibited target cells. The results show that EGCG has an affinity for being a CD4 molecule receptor. The bond between EGCG and the molecules will block the bond with HIV. This is in

accordance with the results of the study¹⁶ that *Epigallocatechin gallate* (EGCG), a catechin present in green tea, protects against the attachment of T-cells to HIV.

In PBMC culture of HIV/AIDS (Figure 1), it was found that after administration of 10mg/ml of green tea extract, *syncytium* cells did not form. This shows that green tea extract is able to inhibit formation of the *syncytium* directly. The findings indicate that there is a close relationship between viral replication and the formation of *Syncytium*. The results of this study agree with Daniel et al.¹⁷ who stipulate that the formation of syncytium or multinucleated giant-cell is the result of infection induced by the human immunodeficiency virus (HIV).

The green tea extract with the highest EGCG content has been reported to inhibit the replication of HIV-1 by targeting several steps in the HIV-1 life cycle, such as interfering with the RT and protease activity, blocking gp120-CD4 interaction by binding to CD4, and inactivating virions.¹⁶ The formation of *syncytium* is affected by the affinity of gp120 to CD4. From the results of this study, it can be concluded that the difference in the ability to form *Syncytium* is not related to differences in gp120 levels on the surface of HIV-infected cells. However, the phenomenon is more influenced by the high affinity of gp120 to CD4. This finding suggests that the amount of gp120 on the surface of cells infected with a virus that does not form *Syncytium* is lower than the surface of the virus-infected cell that forms *Syncytium*. Another possibility is that envelope genes can modulate the expression of cell adhesion molecules on the surface of the infected cell in a certain way to form *Syncytium*.

Green tea extract has the key content of flavonoids, namely epigallocatechin gallate (EGCG). The component is shown to have an *anti-HIV effect* by preventing the binding of HIV glycoprotein (gp) 120 and gp41 to the CD4 molecule on T cells significantly.¹⁸ Despite the finding that the green tea extract inhibition on the gp120 receptor was lower than with the gp41, there was a significant increase compared to no green tea extract. Gp120 is an essential component in the process of the HIV infection, so the ability of green tea extract in binding to gp120 results in resistance to the HIV infection. These results reinforce the findings of Nance¹¹, that tanin or polyphenols contained in green tea can

suppress HIV entry by breaking the formation of the gp41 six helix strand. The results of this study also strengthen the results of Hamza A and Zhan CG research at 2006¹⁹ which stated that EGCG contained in green tea will bind to CD4 and block antibodies and gp120, thereby decreasing HIV infection. The results of the study show that a dose of 10 mg/ml tea extract proved to be more able to bind to gp41 than to gp120. Thus, a 10mg/ml green tea extract can be used as an alternative anti-HIV drug targeted at the gp41 and the gp120.

The results of this study are also in accordance with Fink et al.²⁰ and Steinmann et al.²¹ which state that the physiological concentration of green tea is able to inhibit the attachment of gp120 to the CD4 molecule on T cells. This opinion is in accordance with the results of this study, namely that green extract 10mg/ml with the greatest content of EGCG has the ability to inhibit the formation of *Syncytium* and inhibit the attachment of gp120 and gp41 viruses to CD4 cells in vitro in PBMC cultures. The results prove that the process of HIV infection and fusion to target cells can be inhibited so that HIV/AIDS transmission can be prevented. HIV virus infected human T cells causing immune dysfunction. Patients suffering from AIDS showed the decrease of CD4 reaching less than 200 cell/mm³ or patients experienced opportunistic infection.²²

The conclusion is that green tea extracts, containing EGCG components, may be used as herbal-based anti-HIV candidates through the inhibition of gp120 and gp41 virus. New drug candidates are needed both for antiviral or immunostimulator targeting action for HIV eradication. The discovery of new drug candidates (antivirals), especially for HIV, greatly helps the need for more economical antiretrovirals because they come from natural ingredients.

Conclusion

It was concluded that green tea leaf extract 10 mg/ml has the ability to inhibit the growth of the HIV virus. This *Camellia sinensis* extract could be used as a model of drug development for HIV/AIDS therapy based on the activity in prevention of giant cell and inhibition in gp41 and gp120. This research could be used as basic development of bioactive compound extract of green tea product as an anti-HIV drug.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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