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Doratea: Anti-Cancer Tea From Robusta Coffee Leaves (*Coffea canephora L.)* and Stevia Leaves (*Stevia rebaudiana*)

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ABSTRACT

Abstract: Cancer is an abnormal condition of cell growth that can affect various parts of the body. It is the second leading cause of death in the world with a total of 9.6 million deaths. Many therapies and measures are needed to treat this condition. Cancer treatment involves various methods, such as chemotherapy, radiotherapy and surgery, which are chosen based on the type and stage of cancer. Prevention with a healthy lifestyle is also important to reduce the risk of developing cancer. Doratea is a functional drink with antioxidant content that has the potential to slow the growth of cancer cells. Robusta coffee leaves and stevia leaves contain flavonoids, tannins, saponins, and phenols. This study aims to determine anticancer activity by testing antioxidant and cytotoxin levels with the BSLT (Brine Shrimp Lethality Test) method. Doratea produced from a combination of robusta coffee leaves and stevia leaves, has a %inhibition value of DPPH of 51.187% with a concentration of 5000ppm. As for cytotoxin testing using *Artemia salina* shrimp, the LC50 result is 945.22 mg/L which can be categorized as toxic.

Keywords: Doratea, Robusta Leaves, Stevia Leaves, Anticancer.

Introduction

Indonesia is ranked 8th with the highest incidence of cancer in Southeast Asia (Nita & Indrayani, 2020). Currently, cancer is one of the diseases that causes the most deaths in the world. In 2018 cancer cases reached 18.1 million. This case is predicted to continue to increase until it reaches 29.4 million cases in 2040 (Balatif et al., 2021). The death rate from cancer is around 9.6 million per year and cancer is the

cause of 1 in 6 deaths. This disease is caused by uncontrolled abnormal cell growth in the body. This abnormal cell growth is caused by multiple changes in gene expression, causing an imbalance in cell proliferation and cell death. These abnormal cells will invade and spread to surrounding tissues.

Cases of cancer can be found in both men and women. Cancer is a genetic disease caused by changes in genes that control the function of cell growth and division. The main triggers of cancer are based on several factors such as alcohol consumption, unhealthy diet, smoking, and lack of physical activity. About 1/3 of cancer deaths are caused by unhealthy lifestyles. The problem of cancer certainly does not only affect the patient, but also has an impact on the social, economic community and the country so that preventive efforts from this disease must be done immediately.

Consumption of anti-cancer products can be one of the efforts to prevent this disease. Unfortunately, anti-cancer products in circulation have adverse side effects such as high toxicity. Unfortunately, anti-cancer products in circulation have adverse side effects such as high toxicity. So that researchers want to create an innovation Doratea as an anticancer herbal tea. The content of chlorogenic acid compounds in robusta coffee leaves has anticancer activity. Meanwhile, stevia leaves contain bioactive compounds as anticancer drugs and can also be used as a sugar substitute sweetener. Thus, the combination of robusta coffee leaves and stevia leaves creates herbal tea that is beneficial for the health of the body. We want to introduce Doratea products and spread information about the potential of robusta coffee leaves and stevia leaves as anticancer ingredients.

Research Method

a. Time and Place

This research was conducted at the Basic Chemistry Laboratory of the Poltekkes Kemenkes Denpasar in the process of making Doratea and phytochemical testing, antioxidant activity test, and cytotoxicity test which is located at Jl. Sanitasi No.1 Sidakarya, Denpasar at December 2023.

b. Tools

Tools used in the study, namely vacuum needles, sodium citrate tubes, tourniquet, alcohol cotton, hypafix tape plaster, beaker glass, test tubes, tube racks, measuring cups, stirring rods, gauze, filter paper, drop pipettes, micropipettes, yellow tips, blue tips, tube racks, blender, analytical balance, spectrophotometer.

c. Materials

Materials used in the study, namely robusta coffee leaf powder, stevia leaf powder, 10% NaOH phytochemical screening test, mayer reagent, wagner reagent, dragendorff reagent, distilled water, iron (III) chloride, anhydrous acetic acid, concentrated acetic acid, and aluminum foil, *Artemia salina* shrimp larvae, distilled water, mineral water.

d. The Making of Doratea

Robusta coffee leaves used are old leaves, order 5-9 from the stem, this sampling is because older leaves have greater antioxidant levels. Robusta coffee leaves and stevia leaves are dried by aerating, without drying in the sun to dry and can be pulverized into powder. Doratea was made by combining 1 gram of robusta coffee leaf powder and 1 gram of stevia leaves.

e. Phytochemical Screening of Secondary Metabolite Compounds of Doratea Tea

Flavonoid Identification

Tea is dissolved with ethanol then put into a test tube and added 2 - 4 NaOH 10%, if it gives a yellow color then the reaction is positive.

Alkaloid Identification

Tea is dissolved with ethanol and then filtered to get the filtrate, then the filtrate is divided into 3 each filtrate 1 ml and put into a test tube and then added reagent. In mayer reagent positive reaction formed white or yellow precipitate, in wagner reagent positive reaction formed brown to black precipitate and in dragendorff reagent positive reaction formed orange precipitate. If positive

contains alkaloids if two or three precipitates are formed in the test tube.

Phenol Identification

Tea is dissolved with ethanol and then added FeCl3 reagent 1% where the positive reaction results if there is a purple-black or blue-black color change.

Tannin Identification

Tea is dissolved with ethanol and then added FeCl3 reagent where the reaction results are positive if there is a change in color purple purple-black or blue-black.

Identification of Saponins

Tea is added 5 ml of hot distilled water and dissolved while heated on a water bath and then shaken vigorously if foam is formed and after 10 minutes the foam does not disappear then added HCl 2N foam still does not disappear then the positive reaction is saponin.

Identification of Steroids and Triterpenoids

Tea is dissolved with chloroform and then added 0.5 ml of anhydrous acetic acid, then 1-2 ml of concentrated sulfuric acid is added through the tube wall. If the result obtained is a brownish or violet ring on the border of the two solvents, the reaction is positive for triterpenoids, while if a bluish green color is formed, the reaction is positive for steroids.

f. Antioxidant Activity Testing

Determination of Maximum Wavelength

DPPH solution of 0.5 mM as much as 5 mL was put in a cuvette until full. The λmax of the solution was found and the result of λmax measurement was recorded to be used in the next stage (Hanani, et al., 2005).

Determination of Antioxidant Measurement Stability Time

10,000 ppm tea solution was pipetted as much as 6.75 mL. DPPH 0.5 mM solution was added as much as 2.25 mL, then the stability time without incubation and after incubation at 37ºC and a time range of 5 - 100 minutes with an interval of 5 minutes. Samples were measured at λmax which was known at the previous stage.

Measurement of Antioxidant Potential of Samples

The extract samples were dissolved in their solvents with concentrations of 1000, 5000, 10000 ppm. Tea of each concentration was pipetted 6.75 mL and added 2.25 mL of 0.5 mM DPPH then incubated at 37 oC at the stability time obtained in the previous stage, then measured the absorbance using a UV-Vis spectrophotometer at a wavelength of 518.0 nm. The absorbance data obtained from each concentration of each extract was calculated as the percent (%) of antioxidant activity (Arindah, 2010): = (A control-A sampleA control) × 100%.

g. Cytotoxicity Test Assay BSLT Method

The test was conducted with the BSLT method using *Artemia salina* Leach shrimp larvae (Meyer et al., 1982).

Preparation of *Artemia salina* Leach larvae

Hatching of *Artemia salina* Leach eggs is done by immersing *Artemia salina* Leach eggs in a container containing seawater under the light of a 25 watt lamp. *Artemia salina* eggs used for testing are 48 hours old. Preparation of Test Sample Concentration The concentration of test solution used for each extract was 1000 µg/mL, 100 µg/mL and 10 µg/mL, and negative control Dimethyl Sulfoxide (DMSO) was used.

Implementation of Cytotoxic Test with BSLT method

In the cytotoxic test, a container is prepared for testing, for each concentration of sample extract requires 3 containers and 1 container as a control for each repetition. Furthermore, in each concentration of solution, 10 larvae of *Artemia salina* Leach were inserted. Observations were made for 24 hours on the death of *Artemia salina* Leach larvae, where each concentration was done 3 repetitions and compared with the control. The standard criteria for assessing the death of *Artemia salina* Leach larvae is when *Artemia salina* Leach

larvae do not show movement for several seconds of observation.

Results and Discussions

a. Phytochemical Test

Phytochemical tests were conducted to determine the secondary metabolite content of Doratea. The results of the qualitative phytochemical test are presented in the following table.

Table 1. Phytochemical Test Results of Doratea Tea

Compounds	Results
Flavonoid	(+)
Alkaloid (Wagner)	(-)
Alkaloid (Dragendorff)	(-)
Alkaloid (Mayer)	(-)
Saponin	(+)
Steroid	(-)
Fenol	(+)
Tanin	(+)
Triterpenoid	(-)

Phytochemical test is a qualitative test conducted to determine the presence of bioactive components contained in solvents from leaf extracts. From the results of phytochemical tests that have been carried out, it is known that Doratea positively contains phenols, flavonoids, tannins, and saponins.

Flavonoids are included in the class of secondary metabolites of plants. Flavonoids can generally be found in plants as a mixture so they are rarely found in single form. Consuming certain vegetables, fruits and beverages that contain flavonoids provides a variety of beneficial biochemical and antioxidant effects associated with various diseases such as:

1. Cancer, flavonoids can help inhibit cancer cell growth, induce apoptosis, and inhibit the process of angiogenesis.
2. Neuroprotective, some flavonoids have shown potential in protecting the nervous system and preventing the development of neurodegenerative diseases such as Alzheimer's and Parkinson's.
3. Cardiovascular, Certain flavonoids, such as quercetin and resveratrol, have been linked to protection against cardiovascular disease. They can help improve blood vessel health, reduce inflammation, lower blood pressure, and improve lipid profiles

Flavonoids are indispensable components in various nutraceutical, pharmaceutical, medicinal, and other fields. This is because flavonoids have a variety of

activities such as antioxidant, anti-inflammatory, antimutagenetic, and anticarcinogenic properties.

Saponin is a type of glycoside that is found in many plants and is a complex that has characteristics in the form of foam, so that when reacted with water and shaken it will form foam, saponin is also one of the secondary metabolite compounds contained in plants (Bogoriani, 2008). Saponins can function as antioxidants, activities that inhibit dental caries and platelet aggregation, besides that saponins are compounds that have anti-inflammatory, analgesic, anti-functional and cytotoxic effects (Nwaoguikpe et al., 2010).

Tannin is one type of phenolic metabolite compound that has free radical scavenging activity and can inhibit lipid peroxidase and lipoxygenase. Tannin can also provide protective effects by acting as a free radical catcher and activating antioxidant enzymes. In addition, tannin is able to increase glucose uptake through mediating the insulin signaling pathway. The ability of tannin to capture ROS is then expected to make tannin able to prevent neuronal apoptosis and atrophy of brain cells.

b. Antioxidant Activity Test Result

Antioxidant activity test using DPPH method measured quantitatively with a spectrophotometer.

Table 2. Doratea Antioxidant Activity Test Results

Concentration (ppm)	Absorbance
1000	0.6366
5000	0.2384
10000	0.0910

The antioxidant activity of DORATEA was tested using the DPPH testing method. The antioxidant activity of Doratea showed IC50 = 51.187% with DPPH test.

The antioxidant activity of DORATEA was tested using the DPPH testing method. This DPPH method was chosen because this method is considered simple, easy, fast and sensitive and only requires a small sample. (Afriani et al, 2014). The principle of measuring antioxidant activity using the DPPH method is the measurement of the capture of synthetic free radicals in polar organic solvents, namely ethanol at room temperature by a compound that has antioxidant activity. Testing the antioxidant activity of the sample is done spectrophotometrically using a comparison solution based on its ability in the mechanism of taking hydrogen atoms from antioxidant compounds by free radicals. DPPH solution that reacts with antioxidant compounds through the removal of hydrogen atoms from antioxidant compounds to obtain electron pairs will produce reduced forms of diphenyl picryl hydrazine and non-radical compounds, namely stable DPP Hydrazine. Radical capture antioxidant activity can be determined by the decrease in the uptake (Pokorni, 2001 in Afriani et al, 2014).

Measurement of antioxidant activity was carried out using a spectrophotometer with a maximum wavelength of DPPH of 517 nm. This maximum wavelength gives maximum absorption in the test solution. After the sample solution was mixed with DPPH, the test solution was allowed to stand for 30 minutes before the absorbance was measured. According to Afriani et al (2014). It is intended that the sample solution that has the potential as an antioxidant and the comparison solution reacts to reduce DPPH free radicals until the color changes in the sample solution and the comparison solution

from a dark purple color to a color that decreases in intensity to disappear. The more colorless or the more the color intensity decreases, the higher the antioxidant activity of the sample. Antioxidants in the sample will donate protons (H atoms) to the DPPH radical so that the radical becomes a stable molecule (not radical). The antioxidant activity of Doratea showed IC50 = 51.187% with DPPH test.

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c. BSLT Cytotoxicity Testing Results

The results of cytotoxicity testing using shrimp larvae produced the following data.

Tabel 3. Doratea Cytotoxicity Test Results

Concentration (ppm)	Average (24h)	%Lethality (24h)
0	0.0	0.0
1000	1.7	16.7
5000	2.3	23.3
10000	4.3	43.3

In this test, the result of % DPPH inhibition was 51.187%. The next test is cytotoxicity testing using Artemia salina shrimp, where this test aims to determine the toxic ability to cells (cytotoxic) of a compound produced by plant extracts. From this test, it was found that the sample obtained an LC50 result of 945.22 mg/L which can be categorized as toxic.

After it is known that there is antioxidant content, antioxidant levels are tested with a spectrophotometer to determine the amount of DPPH inhibition from Doratea. In this test, the result of % DPPH inhibition was 51.187%. The next test is cytotoxicity testing using Artemia salina shrimp, where this test aims to determine the toxic ability to cells (cytotoxic) of a compound produced by plant extracts. From this test, it was found that the sample obtained an LC50 result of 945.22 mg/L which can be categorized as toxic. If a material has cytotoxic properties, then the material has compounds that can cancer cells, and can be used to inhibit the growth of malignant tumor cells. Where these results can be interpreted that Doratea has cytotoxic activity that can be used to become an anti-cancer compound.
shrimp, the LC50 result is 945.22 mg/L which can be categorized as toxic.

Conclusion

Robusta coffee leaves and stevia leaves contain flavonoids, tannins, saponins, and phenols. Doratea produced from a combination of robusta coffee leaves and stevia leaves, has a %inhibition value of DPPH of 51.187% with a concentration of 5000ppm. As for cytotoxin testing using Artemia salina

Acknowledgement

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Conflic of Interest

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