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Invitro Assessment of Antiinflammatory and Cytotoxicity activities of *Vitex coffasus* against LPS-induced J774.1 Cells

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Context: The main compounds found in the vitex species are terpenes, flavonoids, and lignans. Flavonoids have shown anti-inflammatory, anti-oxidative, anti-mutagenic, and anti-carcinogenic properties. Virex coffasus (VCS) research was limited, only for larvicides and anticancer compared to other vitex species.

Aim: This study aims to determine the anti-inflammatory and Cytotoxicity activities of *Vitex coffasus* against LPS-induced J774.1 cells.

Method: *:* Initially the Vitex coffasus leaves were extracted by using methanol and water. Next, the extracts were evaluated as Anti-inflammatory by using J774.1 cells functioning as a macrophage and after being triggered by LPS from *Escherichia coli*, these macrophages could produce Nitric oxide (NO). Inhibition of NO was evaluated using Griess reagent. Cytotoxicity activity was determined by using an MTT assay on J774.1 cells that have been previously applied to the Nitric oxide (NO) inhibitory assay. This assay was established to obtain the correlation between NO inhibitory activity and cytotoxicity against J774.1 cells

Results: The extracts effectively reduced lipopolysaccharide (LPS)-induced NO levels in concentration 100 µg/ml. But the extracts showed low cytotoxicity with viability cells over 80 % in the cytotoxicity assay.

Conclusion: From these results, we can conclude that the VCS leaves possess anti-inflammatory effects by reducing Nitric oxide in LPS stimulated in J774.1 cells

Keywords: anti-inflammatory, Cytotoxicity, J744.1, Vitex coffasus

Introduction

Bitti wood (Vitex cofassus) is an indigenous shrub of Sulawesi known as gofasa. Bitti wood (V. cofassus) is used as a building material and a deodorizing agent by the people of South Sulawesi. Several studies have revealed that Vitex coffasus possesses anticancer properties1. Secondary metabolites of flavonoid and alkaloid types identified in bitti wood (V. Coffasus) can inhibit Staphylococcus aureus bacteria.

According to previous work, VCS has anticancer properties. Several studies indicate that cancer has a relationship or association with other disorders, specifically inflammation2-4, which is one of the body's natural defense systems against outside incursions such as microbes and can manifest as rash, fever, and sore3. Cancer development has been linked to

persistent and permanent inflammation. Its emergence might be connected to tumor-related inflammation4, 8-11. Furthermore, infection and inflammation were linked to nearly 25% of all tumor incidents4. Nuraini's (2014)5 research

Using the NO inhibitory assay on LPS-induced J.7441 cells as macrophages, it was found that these cells have characteristics that are similar to those of conventional macrophages in an invitro assay for evaluating inflammation. They have the ability to generate and release lysozyme, and when they kill cells, the antibody identifies the target. These cells came from a BALB/c animal that had an unintentionally developed tumor. When Escherichia coli's LPS activates the J774.1 cells, which perform as macrophages, these macrophages can create NO. Griess reagent was used to assess NO inhibition25–26.

Cytotoxicity cells are crucial for in vitro tests of substances like anti-inflammatory drugs. MTT assay is a colorimetric technique that can assess cytotoxicity. The non-radioisotope character of this test, which is reasonably safe, straightforward, and quick, is one of its benefits. It can be applied in a cell whose preparation is in a non-dissolved medium or single layer27–29, it can be used on a large number of samples, and data from this assay can be simply calculated with a computer program. On J774.1 cells that have previously been used in the NO inhibitory experiment, the MTT assay is used to determine the vitality of the cells. This test was developed to see if NO inhibitory activity and cytotoxicity toward J774.1 cells were correlated.

Based on the description above, this research was conducted to determine the anti inflammatory and cytotoxicity in J774.1 cells of *Vitex cofassus* to increase scientific data from medicinal.

Methods

Preparation Of *Vitex off Asus* Leaves Powder

VCS (Vitex cofassus.) leaf samples were collected, washed with running water, and dried in a drying cabinet at a temperature of 40-50oC. The sample is then pulverized using a grinder, and it is ready to be extracted 8

Preparation Of Methanol Extract *Vitex coffasus* Leaves

The fresh leaves were chopped into tiny pieces and macerated with methanol (1:20, v/v) at room temperature (28 2 °C) for 72 hours with intermittent stirring. The extracted liquid was filtered, and the marc was macerated using the same solvent until the extraction was exhausted. The filtrate resulting from maceration is mixed and then evaporated using a rotary evaporator9.

Anti-inflammatory efficacy against LPS-induced J774.1 cells

J774.1 murine macrophage cells were seeded in 100L per well in 96-well plates. They were then incubated for 24 hours in the CO2 incubator. The following day, the sample was processed and separated into two sections, the first of which included LPS and the second of which did not. The plate was taken from the incubator, and the old medium was replaced with a sample that had previously been produced. The plant extract or isolated component was incubated for a further twenty-four hours.

On the third day, a griess reagent was produced by combining sulphanilamide and N-1-naphthylethylenediamine dihydrochloride. Later, it was dissolved in water containing phosphoric acid, and then additional water was added. New 96 plates were filled with about 100 l of Griess reagent.

The cell plates were removed from the incubator, placed in a cooler, and 100 l was transferred to the Griess reagent plate. The absorbents from the sample in the Griess reagent plate were created using a microplate reader and then the percentage of NO inhibition was calculated.

Cytotoxic action against J774.1 cell population

After assessing the microplate reader (NO inhibition test), the MTT assay was performed on the same plate. 10 l of MTT solution (5mg/ml) was added to the plate, which was then placed in the CO2 incubator for 2 to 4 hours. After removing the medium, 200 l of PBS was added. One minute later, the PBS was withdrawn. About 200 l of DMSO was added to the plate in order to dissolve the formazan crystal that resulted from the mitochondrial enzyme dehydrogenase's reduction of MTT. The result was transferred to a fresh plate, and the absorbance was measured at 570 nm using a microplate reader. Finally, the result was computed to determine the cell viability in each sample.

Result

200 g of VCS (Vitex coffasus) leaf extract was macerated in 2.75 L of methanol to achieve a yield value of 10.063%. The outcomes of extracting samples of VCS leaves (Vitex coffasus) are shown in the table below.

Table 1. Yield value of *VCS* (*Vitex coffasus*) leaves extract

Sample	Sample weight (g)	Extract result (g)	Yield value (%)
VCS Leaves	200	12,92	6,46

The result of anti-inflammatory of VCS leaves (*Vitex coffasus*) can be seen in the following table

2 :

Concentratiom (µg/ml)	Inhbition of Percentage (%)		
10	44,80	97.73	89,09
30	64,25	109,25	100,61
100	84,40	118,65	95,76

The result of Cytotoxicity against J.744.1 cells of VCS leaves (*Vitex coffasus*) can be seen in the following table 2 :

Concentratiom (µg/ml)	Celll Viability (%)	
10	88,63	120,89
30	89,66	131,29
100	86,94	114,91

Discussion

According to studies conducted by Faradiba, one of the compounds identified in the vitex coffasus has anticancer properties. The development of cancer was attributable to chronic and persistent inflammation. Its development may be connected with inflammation linked to tumors2-4. In addition, roughly 25% of all cases of cancer were associated with infection and inflammation. 4. In this study, the preliminary anti-inflammatory VCS screening test was carried out. In this study, the NO inhibitory test on LPS-induced J774.1 cells as macrophage revealed that these cells had the same characteristics as conventional macrophage. They can generate and release lysozyme, and when they kill cells, antibody identifies the target.

These cells were extracted from an unintentional tumor-stricken BALB/c animal. After being activated by LPS from Escherichia coli, the J774.1 cells, which serve as macrophages, may create NO. Using griess reagent, NO inhibition was determined. 10-11

Griess reagents were developed in 1879, and these techniques were extensively used to evaluate nitric oxide in biological samples such as urine, blood, and cultured cells. The procedure for griess reagents is as described below: In the beginning, it will generate short-term diazonium salt, which is formed by a reaction between nitrite and sufanilamide to which acid has previously been added, often phosphoric acid. After adding N-naphthyl-ethylenediamine, this short-term salt transforms into non-labile azo agents with a vibrant purple hue. A microplate reader is then used to measure the absorbant or optical density of a high purple color to determine the NO content. The absorbent calculation results are shown in Table 2 and Figure 1. More than 80% of NO inhibitory action was inhibited by the VCS t at 100 gg/ml concentration. The VCS may be a strong source for further anti-inflammatory development.

The anti-inflammatory properties of the plants are due to their flavonoid content. Cytotoxicity of these plants was investigated to find out the correlation between antiinflammatory and cytotoxicity against J774.1 cells. The result of cell viability was above 80 % - 120 % which means that there was no link between anti-inflammatory activity and cytotoxicity in this research.

Conclusion

From these results, we can conclude that the VCS leaves possess the anti inflammatory activity. no correlation between cytotoxicity and antiinflammatory activity in J.744.1 cells

Ethical approval

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Conflicting Interest

All authors declare no conflict of interest.

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