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ORIGINAL ARTICLE

Antioxidant and Anticancer Activities of Ethanolic Extract of *Laportea sp* Fruit

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ABSTRACT
The purpose of this research was to determine activity of antioxidant and anticancer of the ethanolic extract of Laportea sp fruit. The antioxidant activity of the extract was measured by 2,2'-diphenyl-1-picrylhydrazil (DPPH) free radical scavenging assay. The anticancer activity of the extract was studied using 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay method. The ethanolic extract at 2000 µg/mL showed high antioxidant activity and high anticancer activity. The mean percentage of total antioxidant capacity of extract is 79,081%. The mean percentage of inhibition of A549 cell viability is 83.848 %. The data presented in this study demonstrate that ethanolic extract of Laportea sp fruit showed antioxidant and anticancer activities. The present study suggests potential of the ethanolic extract of Laportea sp fruit as antioxidant and anticancer material.
Key words: Antioxidant, Anticancer, Laportea sp

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INTRODUCTION

Timor Island has source of exotics medicinal herbs. It located in the dry tropics region with a rich biodiversity of plants with specific metabolite that is not found in the wet tropics. Most people on the Timor Island are still tied to the traditional medicine and alternative medicine. This is an opportunity to exploration of traditional medicine source. One of goals of traditional medicine exploration is found active ingredient of antioxidant and anticancer. The *Laportea sp* also known as Kese is a indigenous medicinal herb plants from this Island. The fruit of this plant is mainly used medicinally to treat lever disease and cancer by the people on the Timor Island. They are also believed it is able to maintain a healthy body condition. However, the usage of *Laportea sp* fruit as traditional medicine was very rare and the plants already hard to find. Today, we have not found information about antioxidant and anticancer of *Laportea sp* fruit. It is necessary for research to determine the potential of the *Laportea sp* fruit as an active ingredient of antioxidant and anticancer. This experiment was to evaluate the antioxidant and anticancer potential of ethanolic extract of *Laportea sp* fruit. The purpose of this research was to determine activity of antioxidant and anticancer of the ethanolic extract of *Laportea sp* fruit.

METHODOLOGY

Plants Materials and Extraction

The fresh fruits of *Laportea sp* were collected in March 2012 from Buat, So'e area, West Timor, Indonesia. The fresh fruits (100 g) were air-dried and grinded with added 100 mL of ethanol (merck). The ethanolic extracts prepared by macerating the slurry of dried powdered samples in 900 ml of ethanol (Merck) for 72 h. The Ethanolic extracts ware filtered using Whatman filter paper and the solvent was concentrated under reduced pressure using a vacuum rotary evaporator. The dried extracts

were stored at -4 °C until used.

Determination of Antioxidant Activity

The research was conducted August 2012 to December 2013 in Chemistry Laboratory, Department of Chemistry, University of Nusa Cendana. The free radical scavenging property of extracts were analyzed by 2,2'-diphenyl-1-picrylhydrazil (DPPH) assay. This method was adapted from procedures that described by [1-4]. The dried extracts dissolved in methanol until 2000 µg/mL of concentrations. The assay mixture contained 2 ml of the extract and 2 ml DPPH (0.2 mM in methanol). The same procedure was performed on a blank (2 ml of methanol). After 40 min incubation at 25 °C, the decrease in absorbance was measured at λ = 517 nm. The Antioxidant activity was expressed as percentage of Total Antioxidant Capacity (TAC) that calculated by the equation:

TAC_{DPPH} (%) = (A blank – A sample)/A blank x 100%

The percentage of TAC was expressed as the mean ± standard deviation.

Determination of Anticancer Activity (MTT assay)

Anticancer test with MTT method was quantified according to the method of Hong and Liping [12]. Created a series of test solution of ethanolic extract of the Laportea fruits in some concentration. Cell suspension A549 (Human Lung Carcinoma / ATCC CCL-2) with concentration 2000 cells in 100 µL of media. The cells were were placed in 96-well multi-well culture plate. Extract was added after the cells were reached to 50% confluent (24 hours). The MTT test was performed on day 3, by adding MTT (5 mg/ml) 10 µL into each well, and then incubated at 37 ° C for 4 hours. Formajan crystals dissolved by 0.1 N HCl in isopropanol. Optical absorption was measured by using a microplate reader at a wavelength of 595 nm and the percentage inhibition of cancer cell proliferation was calculated by the formula:% inhibition of cancer cell = [1-OD sample /OD control] x 100%.

RESULTS AND DISCUSSION

Antioxidant Activity of the Ethanolic Extract of *Laportea sp* Fruit

Some methods are used to measure the degree of Antioxidant activity by use of a color reaction to assess the level of Antioxidant activity [5, 6]. Determination of Antioxidant activity by DPPH free radical scavenging assay is a effective method of Antioxidant activity screening. DPPH free radical scavenging assay is a easy, rapid, and sensitive method to for direct measurement of radical scavenging activity of a particular compound or plant extracts [7]. Table 1 shows TAC values of ethanolic extract of *Laportea sp* Fruit.

Table 1. The TAC_{DPPH} percentage of ethanolic extract of *Laportea sp* Fruit

Concentration of ethanolic extract of *Laportea sp* Fruit (µg/mL)	TAC_{DPPH} (%)
100	27.849 ± 2.184
1000	62.538 ± 1.464
2000	79.081 ± 1.342

The results showed a concentration dependent activity of Ethanolic extract of *Laportea sp* Fruit in scavenging DPPH free radical. The table 1 shows the percentage of TAC of ethanolic extract of Laportea fruits at 100 µg/mL (27.849%) has no Antioxidant activity. The mean percentage of TAC of ethanolic extract at 2000 µg/mL (79,081%) is higher than that of the other concentration. The level of Antioxidant activity of ethanolic extract of *Laportea* at 1000 µg/mL and 2000 µg/mL are more than 50% of TAC. It showed TAC of ethanolic extract of *Laportea* fruits at 1000 µg/mL and 2000 µg/mL exceeded IC_{50} value of Antioxidant activity. This fact shows **ethanolic** extract of *Laportea sp* fruits has highly Antioxidant activity at those concentrations. Oxidative stress as an effects of free radicals on the tissue plays an important role in the liver damage. Antioxidants are radical scavengers, which protect the human body against free radicals. Antioxidants are vital substances to protect the body from damage caused by free radical induced oxidative stress. Antioxidants by scavenging free radicals, inhibiting and help in preventing the free radical induced diseases [7, 8]. In 2000 µg/mL and 1000 µg/mL, the ethanolic extract of *Laportea sp* Fruit has strong Antioxidant activity and it may be responsible for the lever disease protection.

Anticancer Activity of the Ethanolic Extract of *Laportea sp* Fruit

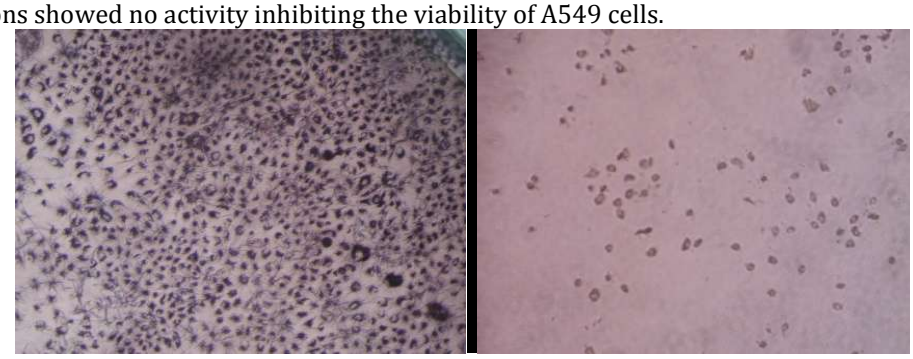
MTT assay is used to test cytotoxicity of compounds against cancer cell lines.The in vitro model was used as one of the method for preliminary study of compounds with potential anti-tumour properties and to test the effectiveness of carcinostatic agents [9]. MTT assay was used to evaluate the cytotoxic activity,

which reduced to an insoluble purple formazan by mitochondrial dehydrogenase. Cell viability was measured by comparison of the purple colour formation between sample and control. The dead cells did not form the colour formation due to the enzyme was not produce [10]. The test results of the viability cells Inhibition of ethanolic extract of *Laportea sp* Fruit are also shown in Table 2.

Table 2. The viability cells Inhibition of ethanolic extract of *Laportea sp* Fruit

Concentration of ethanolic extract of *Laportea sp* Fruit (µg/mL)	The mean percentage of viability cells Inhibition
100	32.238 ± 13.595
1000	49.357 ± 15.07
2000	83.848 ± 9.647

The results of MTT test show the ethanolic extract of *Laportea sp* fruits had activity inhibits the viability of A549 cells line of 83.848% respectively for 2000 µg/mL (Figure 1). In this concentration, the extract can inhibits the viability of A549 cells line more than 50%. It indicate the extract at concentration of 2000 µg/mL showed anticancer activity against to A549 cells line. On the other hand, the other test concentrations showed no activity inhibiting the viability of A549 cells.



a b

Figure 1. A549 Cell Control (a); Ethanolic Extract 2000 µg/mL (b)

The free radicals cause irreversible damage to the components of a cell. The accumulation of damage to the cells induces diseases such as cancer. The Antioxidant plays a preventive role against these diseases by removing the free radicals in biological systems [11].

CONCLUSIONS

In conclusion, the data presented in this study demonstrate that ethanolic extract of *Laportea sp* fruit showed Antioxidant and anticancer activities. The mean percentage of total Antioxidant capacity of extract is 79.081%. The mean percentage of inhibition of A549 cell viability is 83.848%. The present study suggests potential of the ethanolic extract of *Laportea sp* fruit as antioxidant and anticancer material.

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