Pluchea indica Extract as a Potential Source of Nutrition for Accelerate Wound Healing

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ABSTRACT:

In qualitative phytochemical test, beluntas leaves (*Pluchea indica*) extract has proved to have flavonoids. Flavonoids have the potential to be an antioxidant. This is a laboratory experimental study. The beluntas leaves that were used came from botanical garden Manoko Lembang, Balai Penelitian Tanaman Rempah dan Obat, Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture in Bandung, West Java, Indonesia and were determined at the Identification and Determination Laboratory, The School of Life Science and Technology, Institut Teknologi Bandung (ITB). The extraction of 70 % ethanol and the phytochemical test of beluntas leaves were conducted at Aretha Medika Utama Biomolecular and Biomedical Research Center. The determination of total flavonoid levels was carried out by the Quercetin standard using aluminum chloride solvents. The total flavonoid value was obtained from the average value of the measurements after three repetitions. Antioxidant activity was tested by DPPH (2,2-diphenyl-1-picrylhydrazil) Free Radical Scavenging Assay. Absorbance was measured using a UV-Vis spectrophotometer, at λ = 517 nm. In this test the extract was diluted serially, at concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.125 µg / ml. Prestoblue cytotoxicity test was also conducted to determine the effect on viability fibroblast 3T3 BALB C. The beluntas leaves has a total flavonoid of 19.44 mg per 1 gram of ethanol extract. The antioxidant activity included in high category with the IC_{50} value of 21.53 µg/ml. This extract also classified have no cytotoxic activity.

Keywords:
Beluntas leaves, DPPH, flavonoid, wound healing, antioxidant, prestoblue
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Introduction

The wound healing process involves complex cellular, molecular, and biochemical reactions, where the contribution of each cell and molecule to wound healing plays a role in the hemostasis, inflammation, proliferation, and maturation or remodeling phases.[1] The healing ability of a wound greatly influenced by local and systemic factors such as the location, the depth of the wound, and adequate nutrition.[2] The nature can be a source of nutrition.

The extract of beluntas leaves (*Pluchea indica*) has qualitatively proved in phytochemical tests to have flavonoids, saponins, phenols, tannins, terpenoids, alkaloids and steroids or triterpenoids.[3] Flavonoids are polyphenol compounds that have anti-inflammatory effects by inhibiting cyclooxygenase. They also have antioxidant activity and anti-bacterial properties and can increase wound healing by accelerating the rate of epithelialization through the induction of the production of transforming growth factor beta (TGF β).[4] Flavonoids have the potential to be antioxidants due to hydroxyl groups attached to the carbon aromatic ring so that it can bind to free radicals.[5]

Free radicals or ROS (Reactive Oxygen Species) can be produced both in biological systems and exogenously. ROS are known to be able to oxidize lipids, change mitochondrial function,[6] and trigger degenerative disorders such as mutagenesis, carcinogenesis, cardiovascular

disorders, and aging. Antioxidants can significantly intervene in one of the three following oxidative processes, i. e. initiation, propagation or termination.[7,8] Indirectly, flavonoids are able to increase the expression of endogenous antioxidant genes.[9]

The effectiveness of beluntas leaf antioxidant is directly proportional to the total level of flavonoids in it, so in this study the quantifies of total flavonoids and antioxidant activity tests were conducted. The antioxidant activity tests were carried out at various extract concentrations, using serial dilutions. The use of high concentrations of extract can affect cell viability.

Material and Methods

This is a laboratory experimental study.

Ethanol extract of beluntas leaves

Beluntas leaves that were used came from botanical garden Manoko Lembang, Balai Penelitian Tanaman Rempah dan Obat, Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture in Bandung, West Java, Indonesia and were determined at the Identification and Determination Laboratory, The School of Life Science and Technology, Institut Teknologi Bandung (ITB). The extraction using 70% ethanol as the solvents and the phytochemical test of beluntas leaves were conducted at Aretha Medika Utama Biomolecular and Biomedical Research Center.

Total flavonoid level test

The quantification of total flavonoid levels was carried out by the Quercetin standard using aluminum chloride solvents. The total flavonoid test is the determination of the content of flavonoid compound with UV spectrophotometric method. Every miligram of flavonoid in the sample is equivalent to one miligram of quercetin. The principle of measurement is based on color formation. Absorbance was measured using a microplate reader at λ = 415 nm. The total flavonoid value was obtained from the average value of the measurements after three repetitions.

Antioxidant activity test

Antioxidant activity was tested by DPPH (2,2-diphenyl-1-picrylhydrazil) Free Radical Scavenging Assay. The DPPH solution can oxidize compounds in plants. It is purple in colour, and its absorbance was measured using a UV-Vis spectrophotometer at λ = 517 nm. In this test the extract was diluted serially. Working solutions (WS) were at concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.125 µg/ml. The description of antioxidant activity is expressed in the value of inhibition concentration (IC_{50}). The lower the IC_{50} value, the stronger antioxidant activity the extract has. A strong antioxidant activity is categorized as having IC_{50} values of 10-50 µg/ml, a moderate antioxidant activity 50-100 µg/ml, and a weak antioxidant activity > 100 µg/ml.[10]

Data analysis

Data on antioxidant activity in various concentration groups were analyzed statistically using the Kolmogorov-Smirnov One Sample Test and One Way Anova - Tuckey Duncan where the value of α was 0.05. The IC_{50} value of extract of beluntas leaves was also determined using linear regression.

Prestoblue cytotoxicity test

Fibroblasts are cells that are important in wound healing, therefore this extract cytotoxicity test using 3T3 BALB C. This cell planted with a density of 5000 sel/well in 96 well/plate using cell culture medium of Dulbecco's Modified Eagel Medium (DMEM) Fetal Bovine Serum (FBS) 10% and 1% antibiotic-antimycotic (ABAM) then incubated for 24 hours in an incubator with a temperature of 37^{o}C and a CO_2 level of 5%. After 24 hours, prestoblue reagent was added as much as 10µl to each well and incubated for 20 minutes with the same temperature and CO2 levels. The absorbance was measured using a spectrophotometer with a wavelength of 570 nm and 600 nm. In prestoblue cytotoxicity test the extract was diluted serially, at concentrations of 500, 250, 125, 62.5, 31.2, 15.6, 7.8, 3.9 µg/ml. Furthermore, the cytotoxicity of an extract to cells

can be seen from the IC_{50} value. The greater IC_{50} value indicates the extract is not toxic.

Results

Total flavonoid test

The extract of beluntas leaves used for the total flavonoid test was as much as 1 g. The total levels of ethanol extract of beluntas leaves with a quercetin ratio, can be seen in Table 1.

Based on these results it can be concluded that in 1 g of ethanol extract of beluntas leaves there are 19.44 mg of flavonoids. When the result is converted into a percentage, in 1 g of beluntas extract there are 19.44% of flavonoids.

Antioxidant activity

The results of the antioxidant activity test on beluntas leaf extract at various concentrations can be seen in Figure 1. Based on these data, it can be seen that the highest antioxidant activity, which is 93.41, is at a concentration of 200 µg/ml while the lowest percentage, which is 43.48, is at a concentration of 3.125 µg/ml.

Data on antioxidant activity in various concentration groups, when analyzed statistically, were homogeneous and normally distributed data. Based on the analysis of the Tuckey-Duncan Post Hoc test, there were groups that had insignificant differences in average antioxidant activities with p values > 0.05, i.e. in groups with concentrations of 12.5 µg/ml and 6.25 µg/ml, whereas in groups with different concentrations there are significant differences ($p < 0.05$). On these data, linear regression was then performed, so that the IC_{50} value obtained from the ethanol extract of beluntas leaves was 21.53 µg / ml.

Prestoblue cytotoxicity test

The results of the cytotoxic test showed that the highest cell viability value of the ethanol extract of beluntas leaves was at a concentration of 3.9 µg / mL, while the lowest cell viability value was at a concentration of 500 µg / mL. The number and percentage viability fibroblast cells after treated using ethanol extract of beluntas (EEB) can be seen in Table 2.

IC_{50} value of beluntas leaf ethanol extract to determine the half-maximum inhibition concentration using PROBIT. The IC50 value obtained was 311.776 µg / mL.

Discussion

There is still around 60 % prevalence of injuries in the oral cavity in the form of periodontitis in Indonesia.[5] The results of this study are expected to support the effort to develop the potential of beluntas leaf extract to accelerate wound healing in the oral cavity. Beluntas leaves have active compounds in the form of flavonoids, saponins, phenols, tannins, terpenoids, alkaloids, and steroids or triterpenoid.[3,11] Flavonoids are useful to accelerate wound healing especially because they have antioxidant activity. Total flavonoids in herbal plants vary greatly. Even plants that have the same Latin names can have different total flavonoids. Several factors such as the environment in which the plant grows, the time of the harvest and the extraction process also affect the total flavonoids. The total flavonoids in this study are still in the range of some previous studies, i.e. 63.9% to 4% per gram of extract.[12,13]

The antioxidant activity test on extracts of beluntas leaves was carried out using the DPPH (2,2-diphenil-1-picrylhydrazyl) method. It is a stable free radical compound in aqueous or methanol solution. This method is used to determine the antioxidant activity shown by an absorption band in methanol solvent at a wavelength of 515-520 nm, which produces purple colour.[14,15] DPPH free radicals can bind to hydrogen atoms from the extract components mixed into it, then react to be their reduced form and are characterized by a reduction in the purple color intensity of the DPPH solution.[16] Antioxidant activity is determined from the IC_{50} value. The lower the IC_{50} value, the stronger antioxidant activity the extract has . In this study the IC_{50} value is 21.53 µg / ml. Therefore, it is included in the category of strong antioxidants. This is the same category as the resulting category in a previous study (Defitiana Wanita et al. 2018; Manamela & Molapo, 2019; Kaya & Aydin, 2019; Hove & Troskie, 2019), even though there is a

slight difference in IC_{50} values. In the previous study, the IC_{50} value was 37.25 µg / ml.[17] The difference in the two results was caused by a difference in the concentrations of ethanol solvents used. In the previous study, the concentration of the ethanol solvent used was 96% while in this study it is 70%. It is known that 70% ethanol extract of beluntas leaves has less water content compared to 96% ethanol extract.[13]

The results of *prestoblue cytotoxicity test* showed that the IC50 value was 311.776 µg / mL. These results indicate that the ethanol extract of beluntas leaves is safe to use up to this concentration because it does not interfere with the cell proliferation cycle. This value can also indicate the potential for a compound to be toxic to cells. According to the National Cancer Institute, an extract is declared to have cytotoxic potential if the IC50 value is < 30 µg / mL, it is said to be moderate cytotoxic if the IC50 value is 30-100 µg / mL and is declared to have no cytotoxic activity if the IC50 value is > 100 µg / mL. According to ISO 10993-5: Biological Evaluation of Medical Devices regarding Cytotoxicity Test in vitro, if the cell viability is less than 70% then a compound is considered toxic.[18,19] From this classification, it can be concluded that the ethanol extract of beluntas leaves at a concentration of 500, 250, 125, 62.5, and 31.3 µg / mL have toxic properties. While the concentrations of 15.6, 7.8 and 3.9 µg / mL are not toxic to fibroblast cells because the average cell viability value obtained is more than 70%.

Conclusion

Beluntas leaf ethanol extract has a total flavonoid of 19.44 mg per 1 gram of extract. The antioxidant activity of beluntas leaf extract is in high category with an IC_{50} value of 21.53 µg / ml**.** This extract also classified have no cytotoxic activity.

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Table 1 : Total Flavonoids of Beluntas Leaf Extract

	Total flavonoid			
	Concentrations in mg/g of extract			
Repetition	1	2	3	Average
	20.25	20.48	17.58	19.44

Table 2: Viability Fibroblast Cell

Treatment	Number viable cell	%
control	5000	100,00
DMSO 1%	4617	92,35
EEB 500 µg/mL	2519	50,38
EEB 250 µg/mL	2697	53,95
EEB 125 µg/mL	2787	55,74
EEB 62,5 µg/mL	3001	60,03
EEB 31,3 µg/mL	3231	64,63
EEB 15,6 µg/mL	3645	72,90
EEB 7,8 µg/mL	4046	80,93
EEB 3,9 µg/mL	4373	87,46

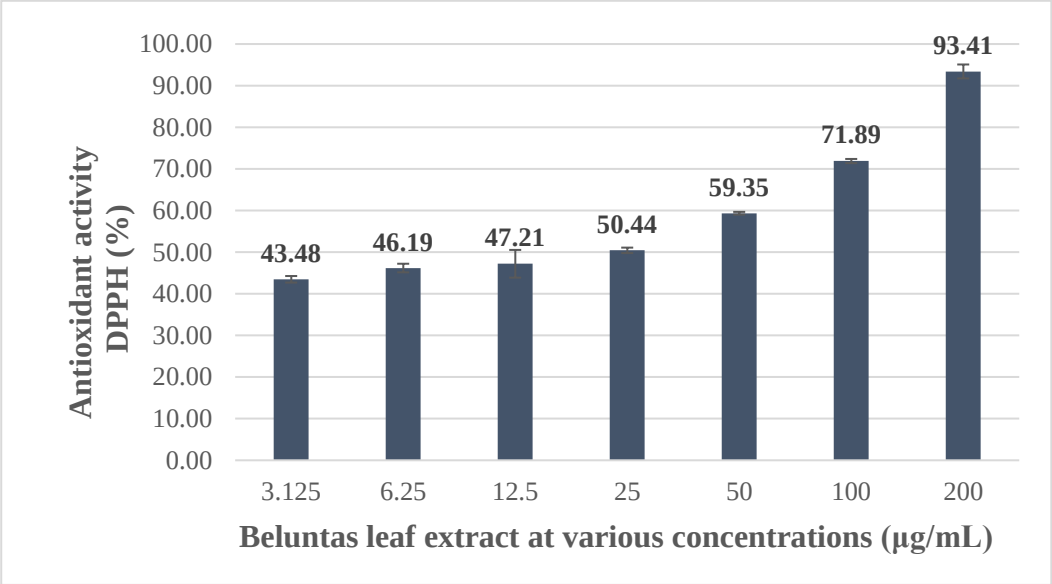

Figure 1: Antioxidant Activity of Beluntas Leaf Extract at Various Concentrations