Cytotoxicity of *Begonia medicinalis* aqueous extract in three cancer cell line

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Abstract. *Begonia medicinalis* or known as benalu batu in Indonesia is a herbal plant that is locally used for traditional medicines. The secondary metabolites such as flavonoids, alkaloids, steroids, and terpenoids have been reported to be found in these plant extracts. The content of flavonoids can lead to anti-cancer abilities while heat-sensitive flavonoid compounds can be extracted by the Ultrasound-assisted Extraction (UAE) method. In this study, the anticancer potential of *B. medicinalis* extracts from the leaves (leaves extract/LE) and stem (stem extract/SE) in three cell lines (Hela, MDA-MB, HT-29) have been performed. Extraction of the leaves and stems was carried out using water as a solvent and the ultrasound-assisted extraction (UAE) method followed by measuring the total flavonoid content (TFC) of each extract. The anticancer potential was obtained from cytotoxic measurements by the MTT method on 3 types of cancer cells incubated with the extract for 24 hours. The value of total flavonoid content (TFC) in the LE was higher than that of SE extracts. Both extracts have the potential as a remedy for the treatment of cancer.

Keywords: *Begonia medicinalis*, Ultrasound-assisted Extraction (UAE), anticancer

1. Introduction

*Begonia* (genus *Begoniaceae*) is one of the largest genera consisting of about 1,800 species. Many cultivars are better known as ornamental flowers [1]. In several countries, its uses as traditional medicinal plants, including for blood cancer [2], consumed for qi balance [3], and fresh juice from the stems of *Begonia apteria* Blume serves to treat intestinal worms [4]. Benalu batu (*Begonia medicinalis*) is one of the *Begonia* plants used as medicinal plants in Indonesia. Mamuju ethnicity, South Sulawesi, one of Indonesia’s provinces, uses all parts of this plant in the treatment of swelling, cysts, cancer, internal diseases, and goiter [5]. A decoction of the leaves from the leaves of Benalu batu (*Begonia medicinalis*) is used by the Wana tribe and the people of Central Sulawesi to treat diseases such as fever, cough, tuberculosis, and cancer [6].

Phytochemical screening of *Begonia* species showed the presence of alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, steroids, carbohydrates, glycosides, and amino acids [6–14]. Flavonoids are produced as secondary metabolites in plants and have a function as plant protection from abiotic and biotic stresses. The strong antioxidant properties of this compound have been exploited experimentally and have beneficial effects on its use as a treatment for several acute and chronic diseases in humans. The biological activity of this compound has been proven in vitro and in vivo and the results show that this compound can function as anti-inflammatory, anticancer, and immunomodulatory. In general, the biological activity of flavonoids occurs because of the nature of these compounds that can scavenge free radicals, regulate cell metabolism, and prevent diseases caused by oxidative stress. The anticancer activity of flavonoids arises because these compounds can act as ROS modulators, play a role in scavenging enzyme activity and cell cycles so that they can induce apoptosis. This role can lead to the suppression of cancer cell proliferation and metastasis [15]. Flavonoids belong to the class of phenolic compounds. There is a linear relationship between the phenolic content in the extract and the antioxidant capacity. The higher the content of phenolic compounds, the stronger the antioxidant activity [16,17]. Metabolite compounds from plant components in the form of leaves, flowers, stems, and roots can be obtained from the extraction process. The extraction method affects the successful isolation of the compound. Some of the flavonoid compounds are sensitive to heating and easily oxidized at high temperatures, so the maceration extraction method was commonly used [18]. The weakness of the maceration method is the use of a large column and place and required a lot of solvents. Ultrasound-assisted extraction (UAE) is a clean method, able to avoid the use of a lot of solvents and large extraction columns, this method saves energy and time, preferred for unstable chemical components as well as...
heat-sensitive components [19]. UAE is one of the extraction methods that has several advantages such as a fast extraction rate, the least interaction with bioactive materials, and is suitable for bioactive materials that are sensitive to hot temperatures. Flavonoid and alkaloid class compounds can be isolated by this method [20].

The biological activity of aqueous extracts of *B. medicinalis* has not been widely studied. Therefore, this study was conducted to determine Total Flavonoid Content (TFC) and examine the potential of the aqueous extract of the benalu batu (*B. medicinalis*) obtained by the UAE method, in its cytotoxic activity in three types of cell lines, which are cervical cancer (HeLa (ATCC® CCL-2™)), breast cancer (MDA-MB-231 (ATCC® HTB-26™)), and colon cancer (HT-29 (ATCC® HTB-38™)).

### 2. Materials and methods

#### 2.1 Plant material

*Begonia medicinalis* from the village of Malino, Soyo Jaya District, North Morowali Regency, Central Sulawesi Province, Indonesia, harvested on March 2021 at the age of 3-4 months.

![Figure 1. Begonia medicinalis also known as benalu batu.](image)

The plant was identified by I Made Mardaka, S.Si, M.Si and a team from "Bali botanic garden-EKA KARYA", LIPI (Indonesian Institute of Sciences). The plant was sun-dried, then the leaves and stems of the plant were separated from the rest of the plant. The sorted leaves or stems are then crushed or ground. The powder obtained was added with water solvent in a ratio of leaf powder: solvent (1:20). The mixture of solvent and the powder was sonicated at a frequency of 20 kHz, an amplitude of 50% for 15 minutes. The result of sonication is filtered to separate debris or powder residue with a solvent containing the analyte of leaf powder and stem powder metabolite compounds. The solution was freeze-dried to separate the solvent so that the extract was obtained from the plant.

#### 2.2 Total Flavonoid Content (TFC)

Total flavonoid content was determined by aluminum chloride colorimetric assay [21] with wavelength modification. Standard solution of quercetin in various concentrations 10-100 μg/ml prepared in 96% ethanol. 50 μl of extracts (1 mg/ml) or standard solution was added to 10 μl of 10% aluminum chloride solution and followed by 150 μl of 96% ethanol. 10 μl of 1 M sodium acetate was added to the mixture in a 96 well plate. 96% ethanol was used as a reagent blank. All reagents were mixed and incubated for 40 min at room temperature protected from light. The absorbance was measured at 405 nm with a microplate reader (ZENIX-320 Microplate Reader). Total flavonoid contents were expressed as mg Quercetin Equivalents (QE) per gram of dry extract.
2.3 Scavenging of Hydrogen Peroxide

The ability of extracts to scavenge hydrogen peroxide was determined according to the method of Ruch with slight modification [22,23]. A solution of hydrogen peroxide (40 mM) was prepared in a phosphate buffer (pH 7.4) and concentration was determined spectrophotometrically at 230 nm (Shimadzu UV-Vis 1700). Samples (5-50 μg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40mM) and the absorbance of hydrogen peroxide at 230 nm was determined after 19 min against a blank solution in phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide of samples and standard compounds was calculated using the following equation:

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\% \text{ scavenged } [\text{H}_2\text{O}_2] = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

where \(A_0\) was the absorbance of the control, and \(A_1\) was the absorbance of samples or standards.

2.4 Cancer cell culture

This study used three types of cancer cells, cervical cancer (HeLa (ATCC® CCL-2 ™)), breast cancer (MDA-MB-231 (ATCC® HTB-26 ™)), and colon cancer (HT-29 (ATCC® HTB-38 ™)). The culture materials used include: Dulbecco, s Modified Eagle Medium (DMEM Gibco, catalog no 11965-092), McCoy's 5A (ATCC® 30-2007), Fetal Bovine Serum Gibco (catalog no 16000-036), Gibco antibiotics (catalog number 15240-062), and trypsin-EDTA Sigma (catalog number 59428C).

The culture method was according to the method issued by ATCC for each cell. The modified medium was used on cervical cancer cells (HeLa (ATCC® CCL-2 ™)), and breast cancer (MDA-MB-231 (ATCC® HTB-26 ™)) with the use of DMEM. At the time of extract treatment, cells were prepared in TC plate 96 well.

2.5 Cytotoxicity assay

The cytotoxicity is measured by using MTT assay, to measure the LC\(_{50}\) of the extract against cells. The materials used are the CellQuanti-MTT ™ Cell Viability Assay Kit, Bioassay system (catalog number CQMT-500). Cells in TC plate 96 well were given extracts with varying concentrations. Incubation was performed for 24 hours. After the incubation was complete, cell viability was measured by the CellQuanti-MTT ™ Cell Viability Assay Kit method.

2.6 Data Analysis

The data obtained in this study are the average of triplicates ± standard deviation. Antioxidant activity, scavenging of \(\text{H}_2\text{O}_2\) value of IC\(_{50}\) data is calculated with probit analysis in the IBM SPSS Statistics 25. Anticancer activity value of LC\(_{50}\) data is calculated with probit analysis in the IBM SPSS Statistics 25.

3. Result

Total flavonoid content (TFC) of the leaves (leaves extract/LE) and stem (stem extract/SE) with 5.66 ± 0.002 and 4.26 ± 0.004 mg Quercetin Equivalent / gram dry extract, respectively.

Scavenging of hydrogen peroxide using ascorbic acid as standard, the IC\(_{50}\) (µg/ml) was 12.39±0.870 from ascorbic acid, 5.216±0.031 from SE, and 6.401± 0.04 from LE.
Figure 2. TFC from B.medicinalis leaves and stem extract (left fig.), and H$_2$O$_2$ free radical scavenging activity of B.medicinalis extracts and ascorbic acid (right fig.).

The aqueous extract is effective as an anti-cancer as shown in LC$_{50}$ (μg/ml) concentration for LE treatment in HeLa, MDA-MB, and HT-29 are 289.69±0.088, 1753.57±0.096, 154.17±0.459 respectively. As for SE treatment in HeLa, MDA-MB, and HT-29 are 1278.29±0.141, 1085.75±0.207, 540.84±0.346 respectively. Cisplatin has been used as control medicine with LC$_{50}$ (μg/ml) concentration in HeLa, MDA-MB, and HT-29 are 41.007±0.364, 104.418±0.46, and 2.98±0.479 respectively.

4. Discussion

Flavonoids can be obtained from plants using the Ultrasound-assisted extraction (UAE) method. The recovery is promising because some flavonoids are sensitive to heat and this can be avoided with the use of UAE [24–26]. Different parts of the plant provide different bioactivities and compounds. The ability of a plant as a medicine is related to the effect and ability of the plant to treat, relieve symptoms and heal pathological damage. Plant parts are commonly used from roots, bark, leaves, and fruit.[27]. Each part of the plant provides a different medicinal effect. In F.deltoidei plants, different parts provided varying antioxidant activity. The results of the study using these plants showed the highest total flavonoid content and antioxidant activity in senescence leaves, followed by fresh leaves, unripe fruit, ripe fruit, and stems. [28]. High-value phenolic compounds can be obtained from T. montanum. This compound can be obtained by extracting all parts or parts of plants with polar solvents[29].

In this present study, total flavonoid content and radical scavenging activities were measured in both LE and SE extracts by aluminum chloride colorimetric assay [21]. Aluminum chloride colorimetric estimation is commonly used to quantify the flavonoid content of plants [21,30]. Total flavonoid contents can be determined by reaction with sodium acetate, followed by the development of colored flavonoid-aluminum complex formation using aluminum chloride in an alkaline condition which can be monitored spectrophotometrically. The results showed that the value of TFC on the leaf extract was higher than that of the stem extract, so the content of flavonoid in the leaf extract was higher than stem extract.

Herbal preparations and their phytochemicals, with their biological activity as scavenging radicals or antioxidants, can prevent oxidation processes and affect cell signaling pathways regulated by redox reactions. This activity can modulate T cells and affect macrophage activation in the immune response, besides that its anti-inflammatory activity can also be used as a treatment for cardiovascular disease, neurodegenerative disorders, infections, malignant tumors, and the formation of an immune tolerance system [31]. The antioxidant ability of phytochemicals, such as flavonoid compounds, can be measured by methods such as radical scavenging on hydrogen peroxide (H$_2$O$_2$). H$_2$O$_2$ itself is not very reactive, but it can produce hydroxyl radicals in cells and can penetrate biological membranes so that it sometimes becomes toxic to cells [23].

In this study, the ability of H$_2$O$_2$ scavenging from LE and SE, when compared with ascorbic acid as standard, the LE and SE showed higher scavenging activity because the value of IC$_{50}$ of the LE and SE
extract was lower than ascorbic acid. The lower the value of IC$_{50}$ means higher activity as an antioxidant. Molyneaux (2004) describes the IC$_{50}$ value, the strength of antioxidant activity is categorized as strong (IC$_{50}$ < 50 ppm), active (IC$_{50}$ = 50-100 ppm), moderate (IC$_{50}$ = 101-150 ppm), and weak (IC$_{50}$ = 151-200 ppm) [28]. Both extracts showed strong antioxidants. The presence of phenolic hydroxyl groups in flavonoids gives these compounds the ability to stabilize free radicals so that they can directly scavenge ROS. Flavonoids tend to possess antioxidant abilities because they can indirectly activate antioxidant enzymes, suppress pro-oxidant enzymes, and stimulate the production of antioxidant enzymes and phase II detoxification. These activities affect the anticancer effect of flavonoids [15]. Phytochemicals found in Begonia species include alkaloids, flavonoids, tannins, and saponins [7]. Phytochemical studies of flavonoids in Begonia flower show that the main components found in ethyl acetate and water extract are flavonoids such as quercetin and kaempferol aglycones or glycosides (mono and diglycosides) [13] and this component possesses antioxidant activity [32]. In the Begonia species, flavonoids were divided into two chemotypes, i.e. flavonol containing type and C-glycosylflavone containing type. For flavonol type, almost all species contain quercetin 3-O-rutinoside as the major flavonoid [1]. Benalu batu was identified as _B. medicinalis_ Begonia section _Petermannia_ (Klotzsch) de Candolle (1859: 134) [33] and flavonoid elucidated from this plant is flavan-3-ol [18], and those compounds showed anticancer activity [34,35]. Other evidence gathered, ethanolic and methanolic extract of this plant showed anticancer activity [6,36]. The water or aqueous extract is not yet examined.

![Figure 3. Percentage of cell death in Hela, MDA-MB, and HT-29](image)

In this present study, anticancer properties were measured by cytotoxic method (MTT assay) on three types of cancer treated with plant extracts which were incubated for 24 hours. The MTT is a dye and is chemically known as 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide. The percentage of cell death was measured and the anticancer activity reported as the value of LC$_{50}$. The lower the value of LC$_{50}$ the higher or more effective as anticancer. The LC$_{50}$ value of both extracts in three types of cancer are higher than cisplatin as control medicine, this means that both extracts have lower anticancer abilities than cisplatin. It could be because cancer drugs are developed specifically and have protein targets, while crude extracts still have many components that can be synergistic or antagonistic in their bioactivities [37–41]. And the mechanism could be investigated further in other studies.
5. Conclusion
The UAE method can be used for the extraction of flavonoid compounds from *Begonia medicinalis*. The TFC value of the leaves extract was higher than stems, and both obtained high antioxidant activity. The aqeous extracts of the leaves and stems of *Begonia medicinalis* have cytotoxic activity in three cancer cell lines. We hope that the research can be continued on the elucidation of active compounds in this plant as cancer therapy.

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