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Anticancer potential of kebar grass (*Biophytum petersianum*), an Indonesian traditional medicine

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Abstract. *Biophytum petersianum* Klotzsch (kebar grass) has been used in Indonesia as traditional medicine. The objective of the current study was to evaluate the cytotoxic activity against several human cancer cells. The plants were collected from Purworejo, Central Java, Indonesia and extracted using methanol and dichlormethane. The extracts were analyzed for its antioxidant activity using DPPH (2,2 diphenyl-1-picrylhydrazyl) method. Cytotoxicity was examined against human acute lymphocytic leukemia cell (CCRF-CEM), multidrug resistance human acute leukemia cell (CEM/ADR5000), human cervical cancer cell (HeLa), human pancreatic carcinoma (Mia-PaCa2) and breast cancer cell (MCF-7) using colorimetric assay for assessing cell viability. The results indicated that methanol extract exhibited higher antioxidant activity as compared to dichlormethane extract. Both extracts exhibited moderate cytotoxicity against several human cancer cells, such as those of CCRF-CEM, CEM/ADR500, Mia-PaCa2 and MCF. This finding was the first report suggested that kebar grass from Purworejo, Central Java, Indonesia was potential as antioxidant and anticancer. Further comprehensive studies on the mechanism of actions are necessary to support this finding.

Keywords: anticancer potential, kebar grass, indonesian traditional medicine

1. Introduction

Currently, plant research has developed significantly around the world due to its potential to cure many diseases. Indonesia has been enriched with biodiversity, among which are medicinal plants. Their therapeutic potential has been evaluated against various diseases. It was provided mainly by its secondary metabolite content, which has been proven scientifically, providing basis in several traditional medicine system.

In recent time, the research has been shifted to find chemotherapeutic agents from natural products. Many plant species and their bioactive compound had anti carcinogenic and antiproliferative effects to inhibit the growth of human cancer cells such as lymphocytic leukemia, cervical cancer; pancreatic carcinoma and breast cancer. [1].



Immunosuppressive factors can be secreted by cancer cell and modified the host immune response. Thereby immune responses are suppressed and causing the development of cancer cell. Moreover, although the chemotherapy and radiation therapy are useful to inhibit the growth of cancer cell, the treatments decline the patient immunity [2]. Plant extracts were able to enhance the immunity in normal and cancerous cells. Natural products, such as herbal extracts have proven potential and could neutralize side effects of cancer in therapeutics. Thus, herbal medicines are potential to substitute synthetic chemical substances in contemporary allopathic medication system which is dangerous for human and environment.

Biophytum petersianum Klotzsch., or kebar grass, has a number of synonyms such as *Biophytum umbraculum* Welw or *Biophytum sensitivum* L. [3]. Its distribution includes tropical countries in Africa and Asia, mainly in Thailand, Malaysia, Indonesia, Philippines, the drier parts of India, Sri Lanka and Nepal [4]. This species belongs to the Oxalidaceae family, locally named as banondit [5] or rumput kebar and can be found in Kebar District, Papua, West Java and Central Java Provinces of Indonesia. This plant is grown wild and not commonly cultivated by people, it disperses naturally. It is an annual herb with unbranched, erect, glabrous or hairy stems from 5 to 20 cm. Leaves are sensitive, pinnately compound, crowded into rosette on top of stem, and 5–12 cm long, with 10–18 pairs of leaflets.

The *B. petersianum* plant contains several secondary metabolites such as alkaloid, tannin and steroidal saponin [6]. According to [7], *B. petersianum* contain vitamin E, alkaloids, saponin, tannins, triterpenoids, steroids and glycosides and flavonoid. It has been well understood that vitamin E and flavonoid compounds can act as antioxidant. The phytochemical content of the *B. petersianum* is affected by the area where the plant is grown. Quantitative phytochemical screening on *B. petersianum* shows that those from West Java has the lowest phytochemicals compared to Papua and Central Jawa [7]. Free radicals can be scavenged by flavonoids and be directly donated the hydrogen atoms through several mechanisms. For example, flavonoids can contribute by interacting with many antioxidant enzymes [8].

The phytochemical of *B. petersianum* contains a number of phenolic and polyphenolic compounds, saponin, essential oil, polysaccharides, bioflavonoid, amentoflavone, and cupressoflavone. The study of phytochemistry revealed that the bioactive compound with major pharmacologically active are amentoflavone and a polysaccharide fraction which is effective as anti-inflammatory, immunomodulatory, anti-inflammatory, antioxidant, chemoprotective, anticancer, wound healing and antidiabetic [4].

Some research also revealed the positive correlation between anti-proliferative effects of the plants and its antioxidant, indicating the role of antioxidant in preventing DNA damage lead to carcinogenesis [9]. The most important characteristic of an antioxidant is its ability to trap free radicals 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) scavenging activity, the most common in-vitro antioxidant method based on the ability of antioxidant to stabilize free radical [10].

In this study, we investigated cytotoxicity of Kebar grass extracts against 5 different cancer cell lines, CCRF-CEM, its multidrug cell line (CEM/ADR5000), HeLa, Mia-Paca2, and MCF-7. In addition, antioxidant activity was also evaluated.

2. Materials and method

2.1. Plant collection and identification

B. petersianum were collected from Purworejo (325 m asl), Central Java, Indonesia, 1090 47' 28" - 1100 8' 20" longitude and 7032'-7054" latitude. All parts of Kebar grass were used for this study. Adults plants were selected characterized by their emerging flowers.

2.2. Plant preparation of extract

Whole plant of Kebar grass were used, washed thoroughly with running tap water, dried under sunlight and grinded at 50 mesh. The plant powder was prepared in Postharvest Laboratory of

Indonesian Spices and Medicinal Crops Research Institute (ISMCRI), Bogor, Indonesia. It was then macerated with dichloromethane (DCM) and methanol (MeOH) to obtain viscous extracts, separately in the dark for 3 days. The extracts were dried over anhydrous sodium sulphate and stored in sealed vials at 4°C for further analysis.

2.3. Antioxidant and cytotoxic activity

Analyses for antioxidant as well as cytotoxicity activity were conducted in the Laboratory of Cell Culture, Biology Department, Institute of Pharmacy and Molecular Biotechnology (IPMB), Heidelberg University, Germany.

2.3.1. Antioxidant activity

Antioxidant activity *in vitro* was assessed using DPPH (2,2-diphenyl-1-picrylhydrazyl) method [10]. The stock of extracts solution and α -tocopherol as positive control were arranged in methanol. A series of dilutions were prepared to get final concentration from 2 to 1×10^{-2} mg/ml. A 500 μ L diluted solution were mixed with 1000 μ L of 0.2 mM DPPH in methanol, and then kept in the dark for 30 minutes at room temperature. Absorbance of each reaction was recorded on a spectrophotometry at 517 nm using a blank containing the similar concentration of extracts or α -tocopherol without DPPH. The radical DPPH inhibition was calculated using the formula:

$$\text{I\%} = [\text{A}(\text{control}) - \text{A}(\text{sample})/\text{A}(\text{control})] \times 100$$

DPPH radical scavenging activity (%) of extracts were presented as IC₅₀ values \pm SD of three replications [11].

2.3.2. Cytotoxicity activity

Cell lines and culture conditions MIA Paca-2 cells was maintained in Dubelcco's modified Eagle's medium (DMEM) with Glutamax (Invitrogen/Gibco, Karlsruhe, Germany), supplemented with 10% foetal calf serum (BioChrom KG, Berlin, Germany), 500 U/ml penicillin, 500 μ g/ml streptomycin, 1% sodium pyruvate, 1% L-glutamine and 1% NEAA; HeLa and MCF-7 cells were maintained in DMEM media as mentioned above without sodium pyruvate and L-glutamine. CCRF-CEM and CEM/ADR5000 were maintained in RPMI 1640 media supplemented with 10% foetal calf serum (FCS), 100 U/ml penicillin, and 100 μ g/ml streptomycin. All cells were cultivated at 37 °C, 5% CO₂, and 95% humidity.

The dose-dependent cytotoxicity was analyzed using the MTT assay [12]. Into each well of 96-well plates were added 2×10^4 of HeLa, MIA Paca-2, and MCF-7 cells and 3×10^4 of CCRF-CEM and CEM/ADR5000 were seeded, and incubated for 24 h. The cells were then incubated with test compounds. After 24 h, 0.5 mg/ml of MTT was filled into each well and then then incubated for 3 h. The formazan crystal produced by viable cells was then dissolved in 100 μ L DMSO and incubated for 10 min. The absorption of the formazan was measured at 570 nm using a Tecan Safire II Reader (Tecan Crailsheim, Germany) [13].

3. Results and discussion

3.1. Plant collection and identification

B. petersianum is widely distributed in Indonesia, ranged from Papua, Central Java and West Java. The kebar grass used in this study was collected from Purworejo, Central Java during dry season in June. Purworejo has wet tropical climate with temperatures between 20°C-32°C, air humidity 70%-90%, and the highest average rainfall in January (292 mm) while the lowest one was in September (23 mm). The selected plants grew wildly under the coconut tree.

The characteristics of kebar grass are similar to coconut with one stem without any branches, 10-12 cm in height and leaf consisted of leaflets in pair as shown in Figure 1 [7]. There were slight differences in leaf and flower color. The plants from Papua have light green color leaf with white flower, whereas from Central and West Java possessed dark green leaf and yellow flower [14] [7].



Figure 1. Kebar grass from Papua (left) [14], Central Java (center) and West Jawa (right) [7]

3.2. Chemical characteristics of plant extract

Kebar grass originated from three locations (Papua, Central Java dan West Java) indicated slight variation in phytochemical content quality (Table 1). Kebar grass from Papua and Central Java indicated stronger tannin, phenolic and steroid content characteristics than those from West Java. Furthermore, GC MS analysis result showed variation in chemical composition (Table 2). This variation might relate to environmental condition [7]. Bioactive component was determined by several factors including geographical origin [15]. Moreover, the change of altitude in the growing site affected environmental components such as average temperature, radiation intensity under clear sky conditions, soil characteristic, wind velocity, low and high temperature extremes, snow cover duration and length of vegetation period [16]. For example, flavonoid content on several plants was affected by environmental factors such as altitude and annual temperature [16]. The variation in phytochemical content also reported on *Hypericum perforatum* from different region which have specific environmental condition [17]. Andropholide content of *Andrographis paniculata* from 12 districts in Banten, West Java and East Java provinces, which have different altitude ranged from 65-908 m asl, was varied from 0.29 to 4.44%. The highest content was from Wonokaton District, Pasuruan, East Java (4.44%) at 31 m asl, while the lowest one was from Conggeang Kulon District, Sumedang, West Java (0.29%) at 398 m asl [18]. The diversification in phytochemical traits like essential oil, oleoresin, and fiber also was found in ginger cultivars in India, collected from different agro climatic zones which have different agro-climatic conditions (rainfall, humidity, temperature and geographical features) [18]. It was also suggested that the altitudinal variations of phenolic in *Arnica montana* caused by the increase of UV-B at higher altitude and decreased temperatures[16]. It would trigger biosynthesis of UV-absorbing and antioxidant phenolics in higher plants. It was in line with the study of [19] on two bracken species *Pteridium caudatum* and *P. arachnoideum*. The levels of high molecular weight phenolic of those two bracken species correlated positively with elevation.

Table 1. Phytochemical constituents of *B. petersianum*

Parameter	Locations		
	Papua	West Java	Central Java
Alkaloids	++++	++++	++++
Saponins	+	+	+
Tannins	++++	+++	++++
Phenolic	++	+	++
Flavonoid	++++	++++	++++
Triterpenoids	+++	+++	+++
Steroid	++	+	+
Glycosides	+++	++	++

Note : ++++ = Very strong; +++ = Strong; ++ = Moderate; + = Weak

Source : [7]

Table 2. Phytochemical constituents of *B. petersianum*

Chemical component	Quantity (%)	
	Papua	Java
Vitamine E	3,25	3,22
9,17 Octadecadienal	23,37	22,61
Octadecanoic acid	3,36	3,97
Hecadecanoic acid	9,87	15,67
Ergost-5-en-3-ol	2,50	2,21
1,1-Biphenyl	3,55	3,73
N-(2,4,6-tris (t-butyl)phenyl	5,00	-
Phenol,3-phenyl	-	2,76
(23S)-etylocholest-5-en-3.beta	7,36	-
Trans-stigmasta	-	1,76
Stigmast-5-en-3-ol	-	13,45
1 beta, Acetoxy-3 beta, hydrocylup	-	2,70
Phytol	-	2,53
Neophytadiene	-	5,88
Spiro (2H-1-benzopyran-2)	-	1,68
Epi-psi,-Taraxastanonol	-	2,30
Otochilone	1,79	-
Myrtifolic acid	2,34	-
Mangiferolic acid	1,96	-
9,19-Cyclolanostan-3-ol	3,36	-
D:C-friedo-oleana-7,9 (11)-diene-3	1,89	-
1-etyl-1-isopropenylcyclohexane	3,43	-
1-(fur-3-yl)-1-(3 acetoxo-4-met	2,14	-

Source : [7]

The extract of *B. petersianum* indicated antioxidant and anticancer activity (Table 3 and 4). Antioxidant activity *B. petersianum* methanol extracts from three locations in Indonesia : Kebar, West Papua 500 m asl; Bogor, West Java 250 m asl and Purworejo, Central Java 350 m asl were 27.74 mg/ml, 45.93 mg/ml, and 38.13 mg/ml respectively [7]. Phytochemical analysis in parts of *B. sensitivum* was rich in numerous beneficial compounds including isoorientin, amentoflavone and cupressuflavone,. Extracts and its bioactive compounds have been recognized to have immunomodulation, anti-diabetic, antibacterial, antitumor, radioprotective, chemoprotective,

antioxidant, antimetastatic, antiangiogenesis, wound-healing, cardioprotective and anti-inflammatory activity [8].

3.3. Antioxidant Activity

Table 3 showed that antioxidant activity of MeOH extract was higher than DCM extract. The extract indicated different antioxidant activity. It might be due to different total content of phenolic and flavonoid, in addition to differences in these bioactive phytochemicals composition. According to [7] phenolic and flavanoid content were qualitatively high.

The methanol extract is commonly solvent used to extract natural antioxidative components, particularly from phenolics group. It might relate to the fact that the methanol possesses high polarity. Thus, it gives greater efficacy in extracting polar phytochemicals such as phenolics and flavonoids [20]. In phenolic, –OH groups were responsible for the antioxidant compounds and their methylation reduces their cytotoxicity [21]. Administration *B. sensitivum* extract in mice scavenge superoxide and hydroxyl radicals and inhibited tissue lipid peroxidation in vitro [22]. Furthermore, the application of *B sensitivum* can protect hemopoietic damage caused by radiation through immunomodulation as well as sequential induction of IL-1 β , GM-CSF and IFN-[2].

Table 3. Phytochemical constituents of *B. petersianum*

Extract solvent	IC50 \pm SD (μ g/mL)
Dichlormethane	380.49 \pm 50.04
Methanol	71.49 \pm 18.94
α -Tocopherol (positive control)	21.13 \pm 0.76

3.4. Cytotoxicity

Analysis of cytotoxicity exhibited that both extract showed moderate cytotoxicity against several human cancer cells, human acute lymphocytic leukemia cell (CCRF-CEM), multidrug resistance human acute leukemia cell (CEM/ADR5000), human cervical cancer cell (HeLa), human pancreatic carcinoma (MiaPaCa-2), and breast cancer cell (MCF-7) (Table 4). The cytotoxicity effect of methanol and dichloromethane extract showed no significant difference. For comparison, doxorubicin was used as positive control (control drug) and indicated an IC50 as shown in Table 4. Dichlormethane extract was very potent and kills all cell lines; meanwhile, the ethanolic extract did not show any antiproliferative effects [23].

In this research, dichlormethane extract showed a moderate to completely inhibitory activity on tumoral cells. It effected proliferation-inhibitory cell cycle progression at the G1 phase and induced apoptosis [24]. *Scrophularia oxysepala* extracted using dichloromethane and methanol inhibited cell growth significantly. Moreover, viability in a dose and time dependent manner did not induce damage to non-cancerous cell line HUVEC [24]. According to [25], the cytotoxicity of cancer cells using methanol extract from *Pitunia punctata* showed a morphological change observed in cancer cells and induced cellular apoptosis, which was confirmed by a biochemical assay verifying increased expression of caspase-3 protein in the cancer cell. Caspase 3, serve as the main executioner of the apoptotic machinery, a point that non-recovering commitment to death. This caspase path was regulated through the conversion of zymogens into the active form in response to apoptotic stimuli or can be reversibly inhibited by the family of apoptotic inhibitor proteins (IAP) [26].

Table 4. Cytotoxicity of *B. petersianum* against human cancer cell

Extract	CCRF-CEM (IC ₅₀ µg/mL)	CEM/ ADR5000 (IC ₅₀ µg/mL)	HeLa (IC ₅₀ µg/mL)	MiaPaCa-2 (IC ₅₀ µg/mL)	MCF-7 (IC ₅₀ µg/mL)
Dichloromethane	58.84±15.63	112±20.84	76.78±10.98	112.1±25.79	57.37±11.93
Methanol	69.94±11.85	124.83±22.96	64.55±11.85	170.0±17.94	86.78±9.37
Doxorubicin (positive control)	0.25±0.02	48.23±8.96	2.21 ± 0.30	19.78 ± 1.78	0.48 ±0.08

Several studies indicated that phenolic compounds such as quercetin, genistein, epigallocatechin gallate and catechin restrained growth of human cancer cell, suggesting the importance of antioxidants towards the antiproliferative effects of cells [9]. Phenolic compounds as phenolic esters inhibited carcinogenesis and reduced cancer development. The rational design of novel chemotherapeutic agent was determined by structure and activity of molecular basis of their anticancer properties [21]. It was expected that the *B. petersianum* extract has several phenolic compounds, one of which is a phenolic derivative anticancer agents. Antioxidant activities in anticancer agents may utilize their beneficial effects by equalizing levels of reactive oxygen species (ROS), hence inhibiting further proliferation of cancer cells while still letting apoptosis to happen [9]. The review of some biological activities of *B. petersianum* suggesting that the plant showed broad biological activities, such as induction of apoptosis, immunomodulatory action, antidiabetic potential, chemoprotective ability, antioxidant, and antitumor activities [27]. The findings of this study constitutes and consistent with the existing study.

3.5. Conclusion and suggestion

We subsequently found that non polar extract of Kebar grass (DCM extract) showed superior activity against CCRF-CEM, CEM/ADR5000, HeLa, MIA-Paca2, and MCF-7 compared with polar extract (MeOH). This study reports the potential of Kebar grass as anticancer. This finding needs further investigation on the chemical structure which responsible to the activity and their complete mechanism of action for application in clinical chemotherapy

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