

# Efficacy of Notgrass Root Extract (*Cyperus rotundas L*) through Inhibition Test Cell proliferation in vitro on Leukemia Cell Line HL-60 and K-562

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## Abstract

This current research aimed at investigating the effect of natural drug (Nut grass root extract) as anti-leukemia candidate for the purpose of contributing to applied science of green technology.

Background: Leukemia constitutes a hematological malignancy, positioned as the 8th highest out of top 10 malignancies across Indonesia. The cell of which is characterized to have an aggressive trait with the instance of uncontrolled proliferation. Its prevalence trends tend to increase. It is worsened by the unawareness of patients, leading to late diagnosis and unsuccessful treatment. Consequently, its morbidity and mortality increase considerably. Nut grass root extract (*Cyperus rotundus L.*) is projected to be a potential natural drug treated as an anti-leukemia candidate by its in vitro inhibition proliferation activity in HL-60 and K-562 leukemia cell lines.

Method and sample: This current research was conducted by means of in vitro experimental method by making use of Nut grass extract tested on leukemia cell line HL-60 and K-562 for anti-proliferative inhibition effects.

Results: The Nut grass root extract (*Cyperus rotundus*) has shown the inhibition proliferation of HL-60 and K-562 cell line, at 20ug / ml, 40ug / ml and 80ug / ml, significantly. By employing Oneway ANOVA test, it has resulted the followings: the Sig 0.000 in HL-60 and 0.000 at K-562,  $p < 0.01$ , (sig = 0.000  $< 0.01$ ,  $R^2 = 0.84$ ) and K-562 (sig = 0.000  $< 0.01$ ,  $R^2 = 0.91$ ).

Conclusion: Alluding to the results of this research, Nut grass root extract (*Cyperus rotundus L.*) potentially inhibits the proliferation of HL-60 and K-562 cell lines. The increase in its intake dose is shown to be inversely proportional to the amount of proliferation. It is proven that the effective dose of Nut grass extract is 80 ug/ml. Further implication of this research is proposing Nut grass extract as the candidate for therapy in leukemia. By that, it contributes the biodiversity of natural product of Indonesia, as well as prevents and controls NCD.

**Keywords:** Leukemia of Cell line HL-60 and K-562, Nutgrass Root Extract, Proliferation cell

## 1. Introduction

Acute Myeloid Leukemia (AML) constitutes a hematologic malignancy, equipped with the insistence of heterogeneous genetic disorder 1,2. It has been characterized typically by an increasing amount of myeloid cells as uncontrolled proliferation of clone cells from the hematopoietic stem cells. Another typicality is that the myeloblasts of which are greater than 20% in peripheral blood or so-called bone marrow 2,3. AML is further noticeable as hematological emergency of cancer. The prognosis is alleged to be poor due to the fact that many patients suffer from relapse and death 4,5, 6..

Furthermore, belonging to the kinds of diseases from a group of non-communicable diseases (NCD), leukemia sufferers are at risk of endangering themselves and thus susceptible to death. Under the convention stipulated by World Health Organization (WHO), since 2011, more than 190 countries have been in one and ultimate stance and commitment to diminish the vulnerability of NCD diseases. Reacting to its global mechanisms, there has been a global action plan to prevent and control NCD through the assertion of "Global action plan for the prevention and control of NCDs Year 2013-2020" 7,8,9. The incidence rate of this sort of cancer is estimated to rise in trend, elaborated as follows. To be particular in the United States, 1,638,910 new cases of cancer are the apparent incidence; 577,190 deaths have been reported due to cancer; new cases of leukemia 47.150 have been detected, worsened by 23,540 deaths; 13,780 new cases of AML and 10,200 deaths have contributed to the long list of sufferers (Siegel et al, 2012). Recorded from 2005 to 2008, in the General Hospital Dr. Saiful Anwar of Malang, bone marrow aspiration (BMP) was undergone by around 385 hematological patients. 250 patients have been diagnosed to suffer from acute leukemia (Database, 2008). To deal with this incidence, chemotherapy has been the intended treatment. Notwithstanding, this sort of leukemia treatment is deemed relatively costly. Besides, its side effects are known to be

spacious, exacerbated by the high possibility of relapse. Forthcoming the era of individualized therapy, human genome approaches and technologies leading to DNA sequencing are of much required, together with gene expression and proteomic. It has been believed to contribute great expectations, bringing about the implementation of genotype-phenotype data. Ahead of cancer therapy, some stages of processes are evident following clinical policy<sup>10</sup>. Basing on the combination of rational drugs towards the target with a variety of cellular pathways, neoplastic therapeutic approaches are imminent. Even more, its existence supports cancer cell survival<sup>10,11</sup>. The challenge in most developing countries, not excluding Indonesia, is that leukemia therapy encounters some sorts of obstacles caused by the undeniable socio-economic status and behavior of parents in responding to the urgency of therapies in their children<sup>12</sup>. Accordingly, herbals are potentially seen as an alternative therapy to develop further. This is not excusable, as Indonesia constitutes a country possessing second largest worldwide biodiversity<sup>13</sup>. *Cyperus rotundus* Linn (Nut grass, family Cyperaceae), the potential traditional medicine, is broadly dispersed in a number of tropical and sub-tropical areas across the world<sup>13, 14</sup>. The compounds of Nut grass root extract function as anti-oxidant and anti-cancer that are ostensible in most pharmacological activities<sup>14,15,16</sup>. Originally, HL-60 cell line is taken from a female patient suffering from Acute Myeloblastic Leukemia (AML) and K-562 Chronic Myeloblastic Leukemia (CML), specifically on the path proliferation and potency test/optimization on active substance of the extract in inhibiting cell proliferation. The combination of rational drugs against targets, with targets of pathway and bio-molecular approaches, are underpinning the forthcoming neoplastic therapeutic approaches (Ocana et al, 2010).

## 2. Materials and Methods

This current research was conducted by means of an in vitro experiment. The method of which was employing the post-test only control group design. The samples were HL-60 and K-562 leukemia cell line from ATCC. 40 patients were examined for in-depth investigation, under the circumstances that HL-60 and K-562 cell line leukemia have been cultured and subdivided into four groups. Each of which consisted 5 cell line pieces. The four groups of samples are divulged as follows: (1) control group (without any exposure to anything), (2) treatment group 1 (exposure to Nut grass root extract *Cyperus rotundus* L.) with the dose of 20 mg / mL), (3) treatment group 2 (exposure to tuber extract sedges with the dose of 40 mg / mL), and (4) treatment group 3 (exposure to tuber extract sedges with the dose of 80 mg / mL)<sup>15,17</sup>. For the purpose of investigating the upshot effects of AML blast proliferation, carboxyfluorescein succinimidyl ester (CFSE)-label AML blast were cultured in the presence, in the period length of 48 and 96 hours before being read by flowcytometry (BD FACS Calibur)<sup>15,17,22</sup>.

## 3. Results and discussion

The measurement results on the amount of cell proliferation given sedges tuber extract at doses 20ug / ml, 40um / ml and 80ug / ml and the control without treatment are presented in the following table:

**Table 1.1:** Average Score Changes of HL-60 Cell Proliferation

| Group                 | Number of Growth |           |
|-----------------------|------------------|-----------|
|                       | Mean             | SD        |
| Control 48 hours      | 15975.50         | 709,960   |
| Dose of 20 ug/ml 48 h | 15146.00         | 610,927   |
| Dose of 40 ug/ml 48 h | 14964.00         | 562,829   |
| Dose of 80 ug/ml 48 h | 7875.00          | 226,078   |
| Control 96 hours      | 16535.50         | 69,481    |
| Dose of 20 ug/ml 96 h | 17359.50         | 51,978    |
| Dose of 40 ug/ml 96 h | 15482.25         | 143,314   |
| Dose of 80 ug/ml 96 h | 14575.75         | 2,839,085 |

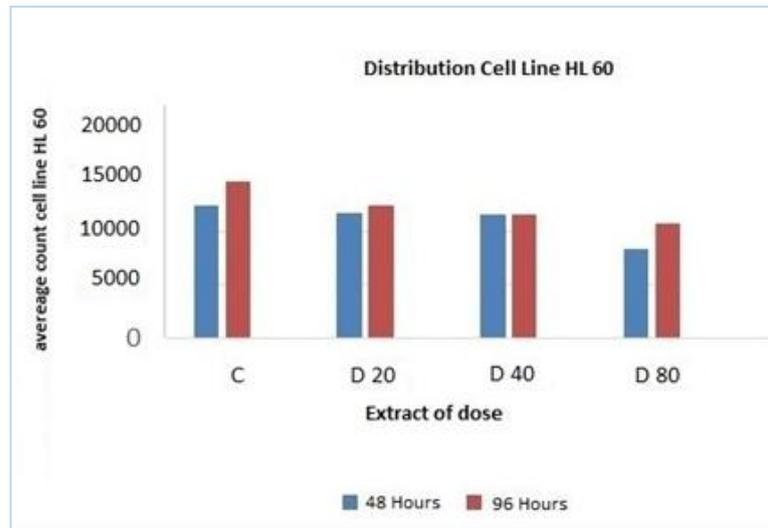
(Source: primary data, 2015)

**Table 1.2:** Average Score Changes of K-562 Cell Proliferation

| Group                 | Number of Growth |         |
|-----------------------|------------------|---------|
|                       | Mean             | SD      |
| Control 48 hours      | 12493.50         | 241,706 |
| Dose of 20 ug/ml 48 h | 11700.75         | 190,162 |
| Dose of 40 ug/ml 48 h | 11536.50         | 61,414  |
| Dose of 80 ug/ml 48 h | 8412.25          | 221,595 |
| Control 96 hours      | 14662.50         | 184,211 |
| Dose of 20 ug/ml 96 h | 12403.75         | 34,180  |
| Dose of 40 ug/ml 96 h | 11632.00         | 94,970  |
| Dose of 80 ug/ml 96 h | 10753.75         | 96,907  |

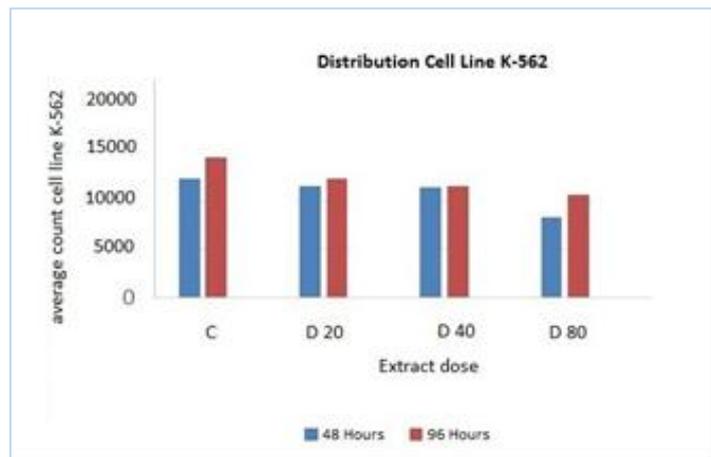
(Source: primary data, 2015)

According to the table 1.1. and 1.2. above the shows that the average amount of which has the most number of cell growth of cell growth at most in each observation was the control group and had a mean number of cell growth at least in each observation is treatment groups by doses of 80ug / ml. the influence of extracts of tubers sedges is starting to look where the number of cell line HL-60 and K-562 to be more decreased after being given treatment tuber of nutgrass root extract started at a dose of 20 ug / ml, 40ug / ml, and 80ug / ml compared with controls without the treatment. In order to clarify decrease in the number of cells can be in the form of graphs that can be seen in graph 1.1; 1.2



(Source: primary data, 2015)

Fig1: Distribution graph dose of 20 ug/ml of nutgrass root extract (Cyperus rotundus L.) HL-60 of cell line



(Source: primary data, 2015)

Fig2: Distribution graph of nutgrass root extract (Cyperus rotundus L.) of K-562 cell line

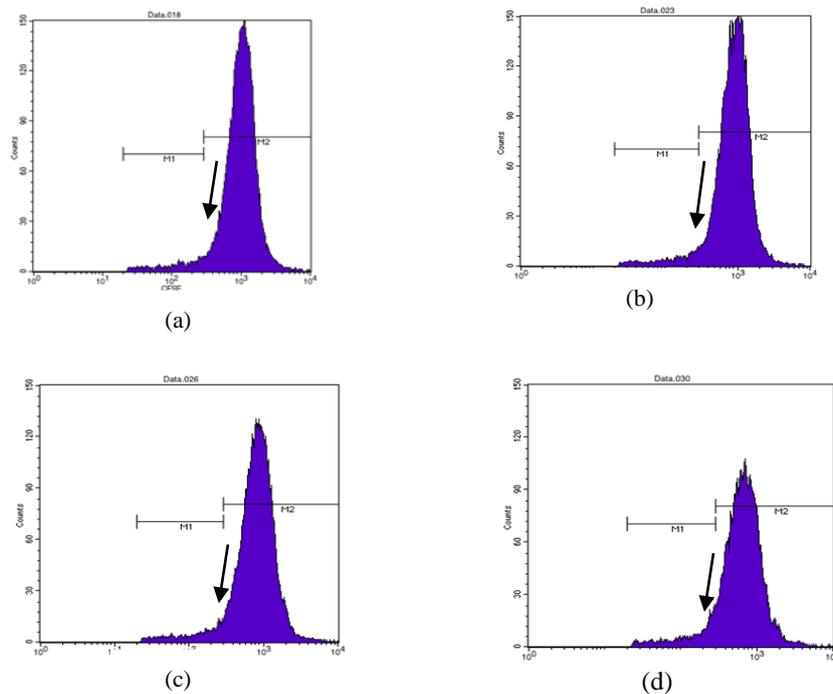


Figure 1.3: Overview on Histogram of Cell Line in control for 96 hours

**Description:**

The Figures have presented cultured cells with histogram picture of Nut grass root extract (*Cyperus rotundus* L.) in the control group (a), the dose of 20 ug/ml (b), the dose of 40 ug/ml, and the dose of 80 ug/ml (Proliferation showed by arrow sign)

They show cultured cells with histogram picture of Nut grass root extract (*Cyperus rotundus* L.) in the dose of 20 ug/ml (b) which began to decrease in the number of significant proliferation and (d) at 80ug / ml dose of Nut grass root extract of *Cyperus rotundus* L with a very significant proliferation.

HL-60 cell line has been extensively used in an intensive number of research models, with varying aspects in the regulation of proliferation's gene expression. The major cause of molecular injury has been said to be vastly contributed by an overproduction of reactive oxygen species. It has been further implicated in the leukemia pathogenesis [14,16]. Antioxidant molecules have been naturally sourced by Nut grass root extract (*Cyperus rotundus* L.) [17,21, 23]. The result has shown that the inhibition on HL60 proliferation is proven to be more visible than that of K562. This is in line with the characteristics of cell growth detected in acute leukemia.

**4. Conclusion**

Nut grass root extract *Cyperus rotundus* L potentially inhibits the proliferation of HL-60 cell line and K-562 cell line. An increase in dose is inversely proportional to the amount of proliferation. The effective dose is 80 ug/ml. An effective targeting of proliferation pathway against promyeloid leukemia cell line HL-60 results in proliferation inhibitors with Nut grass root extract *Cyperus rotundus* L. Furthermore, it may result in less toxic and more efficacious treatment for AML cell line.

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