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Synergistic Potential Of Bioactive Compounds Of Nut Grass Tuber Extract (*Cyperusrotundus*) As Anti-Leukemia Herbal Medicine

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ABSTRACT

Leukemia treatment strategies have developed rapidly. Both the intensity of the dose and the duration of intensive treatment have been maximized after decades of trials optimizing chemotherapy options, dose intensity, and duration of treatment. The emergence of multidrug-resistant cancer cells (MDR) results in the higher costs needed in the treatment of AML patients. The purpose of this study was to identify and evaluate the potential of active compounds from Cyperus rotundus on the activity of the Acute Myeloid Leukemia (AML) cell line HL-60 APL through inhibition of proliferation, cell cycle, differentiation, and induction of apoptosis in the treatment of AML. The bioavailability of the compound to be taken orally was analyzed by Human Intestine Absorption (HIA+). Potential pathways inhibiting proliferation, cell cycle, differentiation, and induction of apoptosis in the treatment of AML were analyzed using a Structure-Activity Relationship approach with the PASS SERVER program. Pathway analysis was carried out using STITCH and Cytoscape v.3.5.1 programs. The study results showed that nutgrass has six active compounds quercetin, luteolin, apigenin, camphor, sitosterol, and gallic acid, synergizing the main pathway of cell activity (Pa>0.7).Conclusion of Cyperus rotundus extract has a high potential synergistically as an anti-leukemia herbal medicine.

Keywords : Acute Myeloid Leukemia,Cell Activity, CyperusrotundusNut Grass tuber extract, In Silicoanalysis

INTRODUCTION

Nutgrass tuber extract (Cyperusrotundus) has a composition of various active compounds from the antioxidant and anti-cancer groups, a grass plant that grows wild in the tropics and subtropics, and one of the plants that are local wisdom of Indonesia's biodiversity. Medicinal plants are widely used in ancient medicine worldwide to treat various diseases, including antioxidant and anti-cancer activity (Himaja et al. 2014). Cyperusrotundus tuber extract contains 24 bioactive compounds, three of which have target proteins from active compounds that function as

antioxidants and anti-cancer (Elia, et al., 2006), which have the potential to induce apoptosis(Soumaya, et al., 2014).

Acute Myeloid Leukemia (AML) is a hematological emergency with a poor prognosis; delay in diagnosis and treatment can lead to a risk of death within weeks to months. Currently, chemotherapy Cell activity is the mechanism of action of the molecular biology of leukemia cell activity, which has been widely used as an intervention strategy for leukemia treatment. (Sak and Everaus, 2017). Cyperusrotundus tuber extract contains active compounds that are potentially important for the activity of Acute Myeloid Leukemia (AML) cells. Of the 24 active compounds from Cyperusrotundus tuber extract, three active protein compounds, including Apigenin, Quercetin, and Luteolin, have a synergistic mechanism in inducing apoptosis through the P53 and Caspase3 pathways (Niu, *et al*, 2010, Shukla, Gupta, 2010; Tuorkey, 2015).

The bioactive analysis method of Cyperusrotundus tuber extract with In-Silico, aimed at predicting biological activity, was carried out using PASS SERVER Cyperusrotundus tuber extract. A computational approach to exploring the potential of new herbs will accelerate the study of new antioxidants and antileukemics. Computational testing was carried out based on the structure-activity relationship (SAR) approach and pathway analysis. The biological activity of an active compound determined from its structure has high accuracy and can be considered before testing in the laboratory. In Addition, path analysis is instrumental in knowing the mechanism of action of an active compound to facilitate the determination of markers in in-vitro testing. Therefore, this study aims to identify the active compound from Cyperusrotundus and determine its potential in cell activity by inhibiting proliferation, differentiation, cell cycle, and inducing apoptosis for in-silico-based AML treatment.

Treatment of AML using chemotherapy drugs with an individualized therapy approach has been widely developed to achieve complete remission. However, there are still many patients who experience recurrent relapses and death. Chemotherapy treatment has high side effects and does not yet have a specific mechanism for target cells. Even these drugs can kill normal cells, and the emergence of multidrug-resistant cancer cells (MDR) results in higher costs required in treating AML patients. Previous research has analyzed the compound using the GC-MS method, obtaining the results of 24 active compounds. Continued research with the in silico method approach uses a String database to study functional protein-protein interactions in humans. Strings also facilitate the analysis of biological processes involving specific proteins. This tool is widely used to propose new drug-induced pathways. Advanced pathway analysis was carried out using Cytoscape software to determine the most likely pathway of an active compound. Biological processes could be studied that would be disrupted if the drug binds to the target protein. The results of the analysis that have been carried out show that 99% of the active compounds from nutgrass tubers have HIA+ values above 0.9; this indicates that, in general, the compounds can be absorbed by the intestine so that they have the potential to be developed as medicinal ingredients that are given orally. Digestion

then identifies the target protein and its involvement in molecular pathways in cells to determine its potential function in cells and as information material for drug development. The potency of the active compound of nutgrass has also been tested using PASS SERVER (Prediction of Activity Spectra for Substances). In this test, the biological activity profile is predicted to inhibit proliferation, cell cycle, and apoptosis agonist based on the chemical compound formula (structure-activity relationship; SAR). The result obtained is the constant value of Pa (Probability activity). This value ranges from 0-1; the closer to one, the more accurate the prediction. The lower limit for this analysis is the value of Pa> 0.7 because we want to get potential with high reliability when tested in the laboratory. Biological activity is predicted based on the suitability of the building formula with the database of compounds that have specific biological activities. More than 4,000 types of physical activities can be traced in this database, such as pharmacotherapeutic effects, biochemical mechanisms, toxicity, metabolism, gene expression regulation, and transporter-related activities. The test results of potential inhibition of proliferation, cell cycle, and apoptosis agonist showed that nutgrass extract had nine active compounds with values above 0.7. Testing of the mechanism of cell activity through the inhibition of proliferation, cell cycle, and induction of apoptosis is an essential indicator in cancer treatment.

RESULTS AND DISCUSSION

The results of the analysis of the active compound content of the Tumput Nut tuber, followed by the second stage of research, this study used the in silico analysis method, which aims to predict the interaction of Nutgrass tuber extract compounds on the cell line activity pathway HL-60 APL sub-type M3 AML, the technique in silico, using the STICH program. The compounds in nut grass tuber extract interact with proteins involved in the cell proliferation cycle and apoptosis; there are interactions of several protein compounds directly involved as anti-proliferation (negative regulation of cell proliferation), namely Quercetin, camphor, Apigenin, Luteolin. The mitotic pathway (cell cycle) functions as a direct transcriptional regulation of the three compounds. Quercetin, Luteolin, and Apigenin, while the camphor binds to proteins. Induction of apoptosis is played by compounds that have the potential, interaction of quercetin compounds directly on activity and transcriptional regulation, sitosterol, and gallic acid direct activity, apigenin transcriptional regulation is shown in Figure 1. These compounds affect the mechanism of action of several pathways that can be determined by analyzing the target protein. This is based on probability activity (Pa); the compound has computational potential if the Pa value > 0.3. If Pa > 0.7, then the computational prediction will not be much different from testing in the laboratory.

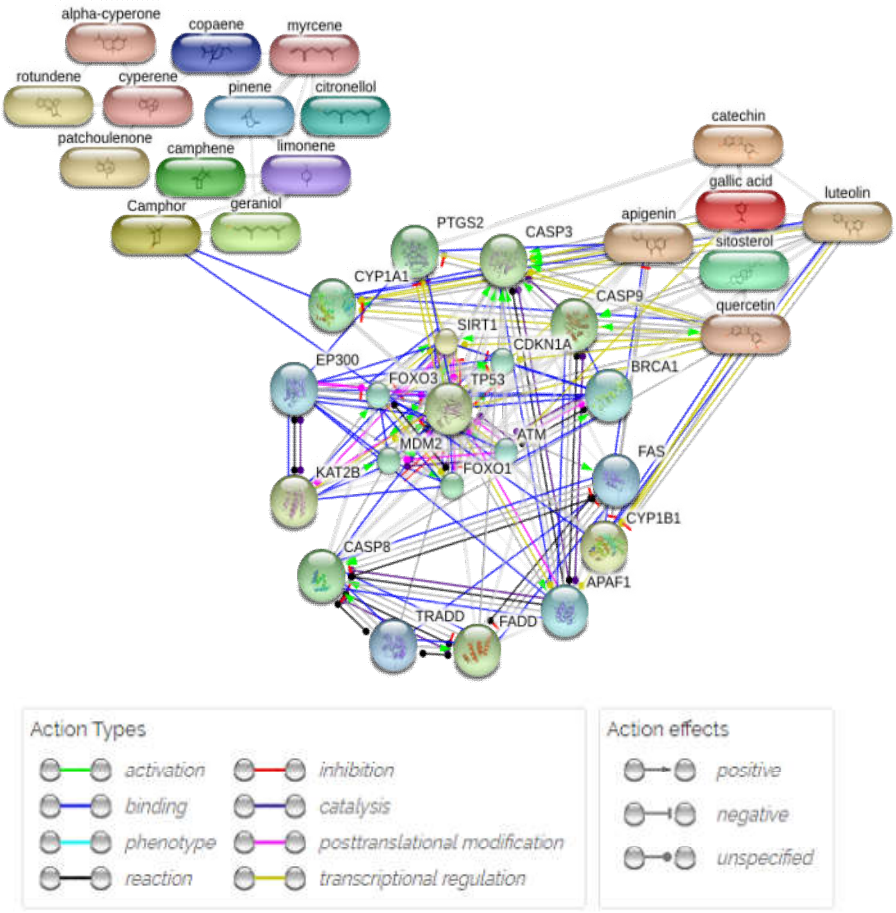

Figure 1. Prediction of the mechanism of the active compound of grass extract with proteins involved in the mechanism (cell cycle, apoptosis, and cell proliferation). Active compounds (apigenin, sitosterol, gallic acid, luteolin, catechin, and Quercetin) have a significant role in treating leukemia**.**

The prediction results of the in-silico nut grass pathway carried out with STITCH v.5.0 software aim to explore compounds' potential and determine the molecular mechanisms that occur in the human body. There are six main compounds from nutgrass that have an essential role in inducing apoptosis, cell cycle regulation, and inhibiting cell proliferation. The results show that apigenin, Quercetin, and luteolin are known to have a synergistic mechanism in inducing apoptosis through the P53 and Caspase3 pathways. In Addition, these compounds also interact with MDM2, which has a role in cell cycle arrest, and protein kinase CDKN1A, which plays a role in controlling cell proliferation (Figure 1). Apigenin is a promising compound for cancer prevention. Human clinical trials examining the effects of apigenin supplementation on disease prevention have not been carried out. However, there is potential for apigenin to be developed as a cancer chemopreventive agent (Shukla, Gupta, 2010). The mechanism of Quercetin as chemotherapy with the mechanism of inhibiting cell proliferation and inducing apoptosis in time and depends on the dose. Furthermore, Quercetin decreased the expression of the anti-apoptotic protein Bcl-2 and

upregulated the expression of the pro-apoptotic protein Bax. Caspase-3 is also activated by Quercetin, which initiates the mitochondrial pathway.

The results of the analysis of the active compound content of the Tumput Nut tuber, using the in silico analysis method, which aims to predict the interaction of Nutgrass tuber extract compounds on the HL-60 APL cell line activity pathway of the M3 AML subtype, the in silico method, using the STICH program. The compounds contained in nut grass tuber extract interact with proteins involved in the cell proliferation cycle and apoptosis; there are interactions of several protein compounds directly involved as anti-proliferation (negative regulation of cell proliferation), namely Quercetin, camphor, Apigenin, Luteolin. The mitotic pathway (cell cycle) functions as a direct transcriptional regulation of the three compounds. Quercetin, Luteolin, and Apigenin, while the camphor binds to proteins. Induction of apoptosis is played by compounds that have the potential, interaction of quercetin compounds directly on activity and transcriptional regulation, sitosterol, and gallic acid direct activity, apigenin transcriptional regulation is shown in Figure 1. These compounds affect the mechanism of activity of several pathways that can be determined by analyzing the target protein. This is based on probability activity (Pa); the compound has computational potential if the Pa value > 0.3. If Pa > 0.7, then the computational prediction will not be much different from testing in the laboratory. It is affected by the cytochrome protein inhibitory mechanism and the activation of caspase3 as an executor of apoptosis.

CONCLUSION

Nutgrass tuber extract (Cyperusrotundus) can contain active compounds quercetin, luteolin, apigenin, camphor, sitosterol, and gallic acid, which have synergy in the main pathway cell activity as herbal medicine anti-leukemia.

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