Research Article

An *in silico* approach toward wheatgrass extract-induced apoptosis of human acute myeloid leukemia cells

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ABSTRACT

Background: Extracts of wheatgrass (*Cyperus rotundus* L.) have the potential to be developed as anti-leukemia agents. Aim: This study was aimed to identify and evaluate the potential of the active compounds in *C. rotundus* in inducing apoptosis in the treatment of leukemia. **Methods:** This study was conducted in several stages: Identification active compounds of *C. rotundus* by gas chromatography/mass spectroscopy analysis, human intestine absorption, prediction of activity spectra for substance, and pathway analysis. **Results:** The result of the study showed that wheatgrass has nine active compounds and 24 compounds capable of performing apoptosis processes (probability activity >0.7). All the compounds can be absorbed by human intestine (HIA >0.9). Pathway analysis showed that the active compounds quercetin, luteolin, and apigenin possessed synergy in inducing apoptosis. The main pathway affected is the mechanism of the inhibition of cytochrome proteins and the activation of caspase 3 as the apoptotic executor. **Conclusion:** Thus, extract of *C. rotundus* has a high potential in inducing apoptosis through cytochrome inhibition and caspase induction, and this is a good candidate for further laboratory testing for the treatment of leukemia.

KEY WORDS: Acute myeloid leukemia, Apoptosis, *Cyperus rotundus, In silico*

INTRODUCTION

Wheatgrass (*Cyperus rotundus*) is a member of the sedge family that grows wild in tropical and subtropical areas, among Indonesia's natural biodiversity of medicinal plants. Medicinal plants are widely used in ancient medicine worldwide to heal various diseases having such roles as antioxidant and anticancer agents.[1] A root extract of *C. rotundus* contained 24 bioactive compounds, three of them target proteins that functioned as antioxidants and anticancer agents and which have potential in the process of apoptotic induction.[2]

Apoptosis is one of the mechanisms that can disrupt leukemia cell activity and that is generally used as a leukemia treatment intervention strategy to death.[3] The root extract of *C. rotundus* contains active compounds that have a potential effect on acute myeloid leukemia (AML) cell activity.[4] From the 24 active compounds in the root extract of *C. rotundus*, three were active protein compounds, apigenin, quercetin, and luteolin, known to hold a synergistic mechanism in inducing apoptosis through P53 and caspase 3.[5-7]

The bioactive method for *in silico* analysis of the root extract of *C. rotundus* using was aimed to predict the biologic activity of the extract, as established using prediction of activity spectra for substances (PASS) SERVER. A computational approach to explore new herbal potentials can hasten the study of new drugs. The computational examination is carried out based on an approach of structure–activity relationships (SARs) and pathway analysis. The biological activity of active compounds determined according to their structure, possesses high accuracy, and can use to consider candidate agents before laboratory testing. Besides, elucidation of the pathway is extremely beneficial in discovering the mechanism of active compounds, making it easier to determine markers *in vitro* examination. Therefore, this study aimed to identify the active compounds in *C. rotundus* and

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identify the potential for apoptosis induction *in silico* for the treatment of leukemia.

MATERIALS AND METHODS

Obtaining *C. rotundus* Samples

Identification of plant specimens was performed at UPT Materia Medica Batu Health Department of East Java Province.

Isolating and Identifying the Active Compounds using Gas Chromatography/Mass Spectroscopy (GC/MS)

Maceration was carried out by inserting 10 parts of *C. rotundus* root powder into a maceration container. Then, 75 parts of the solvent solution were added to the container, which was then closed and left for 24 h at room temperature, protected from light. After incubation, the mixture was stirred repeatedly, allowed to settle and the transparent liquid above the solid layer isolated. Next, the solvent solution was readded until 3 times. The liquid was evaporated using a rotary evaporator until a concentrated extract was obtained. Further, analysis of the extract was carried out by GC/MS.

Analysis of Human Intestine Absorption (HIA+)

Intestinal absorption and metabolism of the active compounds used for treatment were examined. Absorption, distribution, metabolism, excretion, and toxicity (ADMET) was established to identify if the metabolites can be easily absorbed by the human digestion. The parameter used was HIA+. The results showed that 99% of the compounds had an HIA+ value >0.8, which generally indicated that the compounds can be taken orally.

Prediction of the Biological Activity

The prediction of biological activity was carried out using the PASS SERVER program (http://www.pharmaexpert.ru/passonline/). The prediction was done based on the SAR (structure–activity relationship) approach. If the compounds hold the same structure and active group, it is predicted that they have the same activity. PASS SERVER scoring applies constants (probability activity [Pa]), which is Pa, with a threshold 0.7. If the Pa > 0.7, it means that the prediction result computationally will not be much different if it is laboratory tested.[8] The biological activity analyzed was apoptosis agonist and anti-inflammatory.

Prediction of the Target Protein

The target protein for the main active compounds on wheatgrass was analyzed using STITCH. The approach used was a targeted-focused library aiming to find the potential protein which was the target of the new compound group. The principle used was to find the similarity of the functional group and structure between the new compounds and the compounds whose target protein was previously identified. If the target protein was identified, then the work pathway mechanism was analyzed. Thus, the biological process that would be disrupted if the drugs bind to the target protein can be learned.

Prediction of the Protein Interaction Pathway

The interaction between target proteins and the other proteins involved in a particular pathway can be analyzed. The service used was String DB (http://string-db.org). The string database can be used to study the interaction of human functional proteins. String also facilitates biological process analysis involving certain proteins (Szklarczyk *et al.*, 2015). This tool is widely used to propose new pathways induced by drugs. The advanced analysis of pathway was carried out using the *Cytoscape* software to identify the most likely path of an active compound.

RESULTS AND DISCUSSION

Management of AML

Testing of the root extract of *C. rotundus in silico* aimed to predict the biological activity using PASS SERVER. The computational approach to explore the potential of new compounds can hasten the study of new drugs. As noted above, computational testing was done based on the SAR approach and pathway analysis.

The approach of the next neoplasm therapy requires the use of combination therapy of the rational drugs on the target based on the various pathway activities of cancer cells.[9] There have been many studies on the treatment for AML through the apoptosis pathway.[3] Root extracts of *C. rotundus* contain active compounds that are important potential for the cell activity (AML). Taking into consideration that AML patients usually have a poor clinical prognosis, which is primarily associated with chemotherapy or relapse after previous chemotherapy response which fails, and there has been no significant progress in treatment, it is important to develop an innovative therapeutic approach to improve the response and life sustainability of AML patients.[10] Chemoresistance is a major obstacle to the success of therapy against AML, which is associated with apoptotic deregulation. Hence, it is important to identify new chemotherapy targets that can be used to induce apoptosis in AML.[11]

Active Compounds in Wheatgrass

The result of GC/MS showed that there were 24 active compounds in the root extract of *C. rotundus* [Figures 1 and 2].

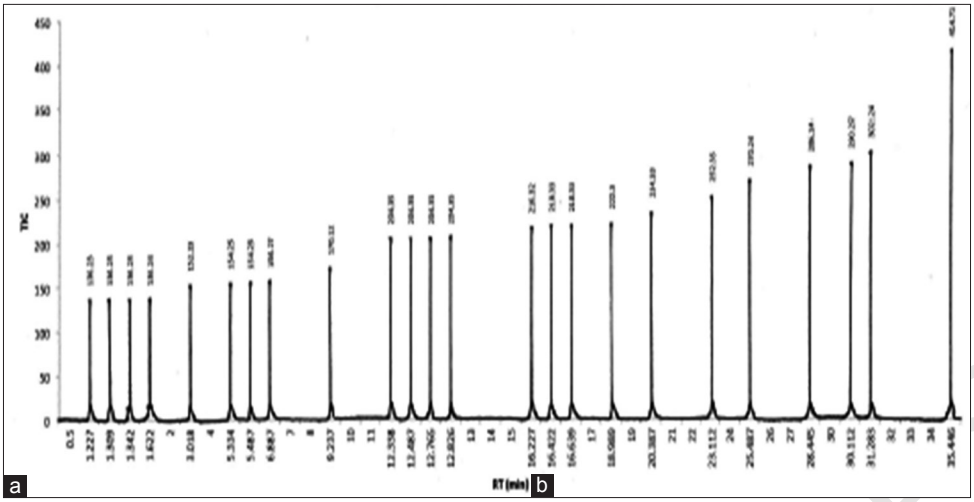

Figure 1: The result of chromatography of the wheatgrass extract. (a) The number of identified active compounds. (b) The molecular weight of the identified active compounds

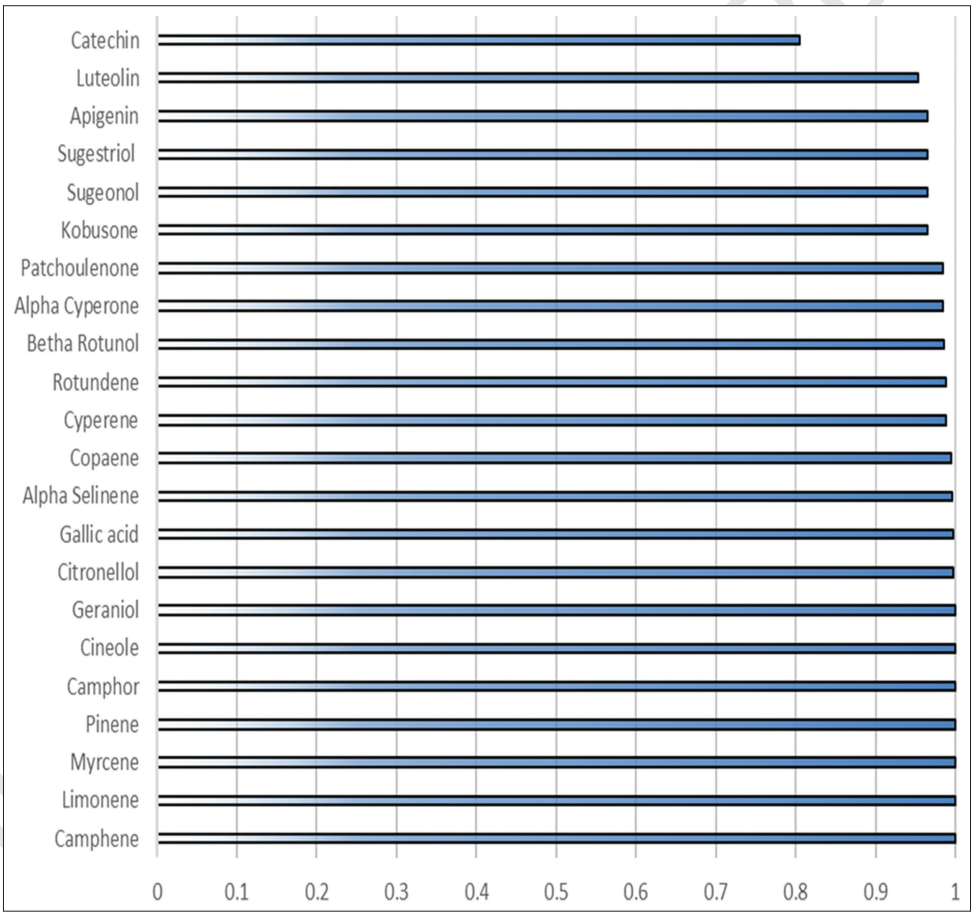

Figure 2: The potential of each active compound that was predicted to be capable of being absorbed by the intestine or digestion, so it can be orally given (value close to 1: can be easily absorbed by the intestine). One active compound, namely catechin, has a value of 0.8 but can be still well absorbed

The 24 active compounds were camphene, limonene, myrcene, pinene, camphor, cineole, geraniol, citronellol, gallic acid, α-selinene, copaene, cyperene, rotundene, β-rotunol, α-cyperone, patchoulenone, kobusone, sugeonol, sugestriol, apigenin, luteolin, catechin, quercetin, and β-sitosterol. These components were different than reported in other studies. For instance, the composition of the essential oils obtained from two wheatgrass extract collected in different locations in South Africa and extracted using hydrodistillation was analyzed using a GC capillary and GC/MS method; α-cyperone (11.0%),

Table 1: The target proteins of the active compounds in the extract of *C. rotundus*

Compounds	Target	Mechanism	Value
Apigenin	P53	Expression regulation	0.15
	CASP3	Activation	0.7
Luteolin	MAPK	Inhibition	0.7
	CASP3	Activation	0.7
	P53	Binding	0.828
Quercetin	CASP3	Activation; expression regulation	0.9; 0.3
	TP53	Activation; expression regulation	0.7; 0.5
	MAPK8	Inhibition	0.8
	SIRT	Activation	0.150
	CDKN1A	Expression regulation	0.255
	CDKN2A	Interaction	0.725

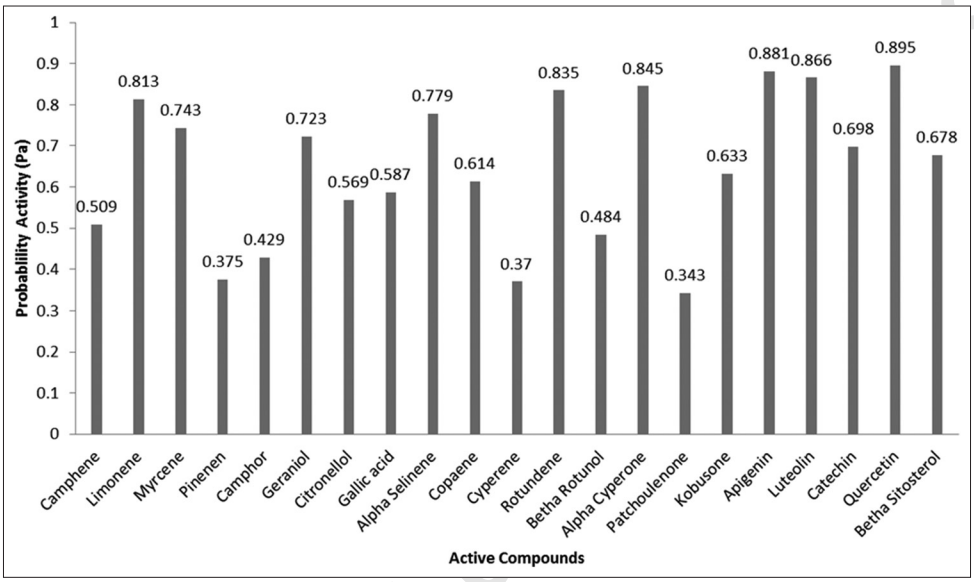

Figure 3: The potential of the active compounds in *Cyperus rotundus* in inducing apoptosis of cancer cells based on the Pa value, ranging from 0 to 1; the closer the value to 1, then the more accurate the predictions. The threshold used to determine the potential bioactive is probability activity > 0.7; this value showed that the prediction will be computationally appropriate to be further laboratory tested

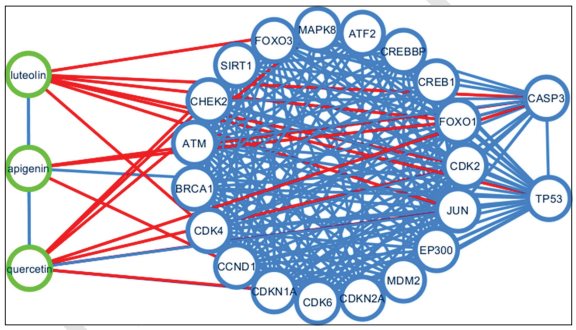

Figure 4: The working mechanism of the active compounds on wheatgrass in inducing the process of cell apoptosis (red lines show the direct interaction with the active compounds)

myrtenol (7.9%), caryophyllene oxide (5.4%), and β-pinene (5.3%) were the main components of sample A, while the main compound found in sample B was β-pinene (11.3%), α-pinene (10.8%), α-cyperone (7.9%), myrtenol (7.1%), and α-selinene (6.6%).[12] Pal and Dutta (2006) found that roots of *C. rotundus* were rich in antioxidants such as sitosterol, cyperene, cyperol, flavonoids, sesquiterpenoids, ascorbic acid, and polyphenols.[13] In addition, Sivapalan (2013) found *C. rotundus* also contains alkaloids, essential oils, triterpenes, and carbohydrates.[14]

The Potential of *In Silico* Evaluation of Active Compounds

Metabolic characteristics such as ADMET can be predicted using bioinformatics based on the physical characters of molecular chemistry. The analysis results showed that 99% of the active compounds on wheatgrass had HIA+ values >0.9, and this shows that the compounds can be generally absorbed by the intestines, so they can be developed as orally delivered drugs.

The target protein and the involvement of the active compounds that were easily absorbed were identified in the molecular pathway in the cell to discover their functional potential and as the information for the development of drugs.

The potential of active compounds in *C. rotundus* was also tested using PASS SERVER. In this test, the biological activity was predicted as an apoptosis agonist based on the SAR. The result obtained was the constants of Pa. The value ranges from 0 to 1, the closer the value to 1, then the more accurate the predictions. The lower limit for this analysis is Pa > 0.7 because it was supposed to get high-reliability potential when it was laboratory tested. The biological activity was predicted based on the build formula with the compound database holding certain biological activity. Biological activities that could be suggested in this database were more than four thousand types of activities such as pharmacotherapeutic effects, biochemical mechanisms, toxicity, metabolism, gene expression regulation, and transporter-related activities. The test result of apoptosis agonists showed that the extract of *C. rotundus* had nine active compounds holding a value >0.7 [Figure 3].

Camphene, limonene, myrcene, pinene, camphor, geraniol, citronellol, gallic acid, alpha-selinene, copaene, cyperene, rotundene, beta-rotunol, alpha-cyperone, patchoulenone, kobusone, apigenin, luteolin, catechin, quercetin, and beta-sitosterol are active compounds *C. rotundus* extract.

The nine active compounds known for their potential in inducing apoptosis were then suggested as to their mechanism *in vivo* [Table 1]. The testing of apoptosis induction is the main indicator of the process of the treatment of cancer. Besides, the activity of the extract of *C. rotundus* contains *in vitro* antioxidant activity and has potential to induce apoptosis.[2]

Apigenin, quercetin, and luteolin are known to have a synergistic mechanism in inducing apoptosis through the P53 and caspase 3 pathways [Figure 4]. Apigenin is a compound supposed to prevent cancer. The human clinical trials examining the effects of apigenin supplementation on disease prevention have not been performed, although there is a potential for apigenin to be developed as a cancer chemopreventive agent.[6] The mechanism of quercetin as the chemotherapy with mechanism inhibiting the cell proliferation and inducing apoptosis in time and depends on the dose. Quercetin decreases the expression of anti-apoptosis protein Bel-2 and manages the protein expression of pro-apoptosis Bax. Caspase-3 is also activated by quercetin, initiating the mitochondrial pathway of caspase-3 dependency to induce apoptosis (Niu *et al*., 2010). The function of luteolin compounds is known to prevent tumor development mainly by deactivating some signaling and transcription pathways that are important for cancer cells.[7]

The activation of p53 is extremely important in the process of treating cancer. Apigenin, quercetin, and luteolin hold the potential for enhancing the expression and inducing the activation of the protein. After the process of activation, p53 causes the activity of cell cycle regulatory proteins such as p21. The protein p21 will inhibit cycling E Cdk2, due to this obstacle, the cell cycle will discontinue at phase G1. This series of processes take place inside the cell; at the same time, p53 also stimulates the permeability of the outer membrane of the mitochondria, causing loss of the potential of the membrane and subsequently followed by the release of a number of pro-apoptotic proteins such as Bax. The process of apoptosis also depends on the availability of caspase-3. This protein holds a crucial role in the process of apoptosis; caspase-3 could be bound to caspase-8 and caspase-9 in performing the function. Caspase-3 activates the process of cell apoptosis both extrinsically and intrinsically.[7] The increase of caspase-3 expression and the activation induced by the active compound are highly beneficial for cancer treatment. Since the clinical condition of AML patients is usually poor, associated with chemotherapy or relapse after the responses of the previous therapy and no significant advances in treatment, it is extremely important to develop innovative therapeutic approaches to improve the response and life sustainability of AML patients.

CONCLUSION

From this study, it can be summarized that there are 24 active compounds identified from *C. rotundus* and only 10 active compounds that purpose in inducing apoptosis. Three active compounds are known to have synergy in inducing the apoptosis pathway, namely quercetin, luteolin, and apigenin. *C. rotundus* extract should likely continue with laboratory verification testing for anti-leukemia activity.

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