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Original Article

Cytotoxic Effects of *Imperata cylindrica* Root Ethanol Extract on the Expression of PKCα, STAT3, and mTOR in A549 Lung Cancer Cell Line

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ABSTRACT

Background: *Imperata cylindrica* or Cogon grass is a medicinal plant that has an anticancer effect. We unravel the cytotoxic effect of Imperata cylindrica root ethanol extract on the expression of PKC α, $STAT_3$ and mTOR in A549 lung cancer cell lines. **Methods:** The cytotoxic effect tests were carried out on the A549 lung cancer cell line after 48 hours post-treatment using 3- [4, 5-dimethylthiazol-2-yl] -2.5 diphenyl tetrazolium bromide (MTT) assay method to discover the IC_{50} dose, continued with apoptosis tests at doses of 150, 300, and 600 µg/mL of I.cylindrica root ethanol extract using flowcytometry, as well as measurements of PKC α, STAT3, and mTOR mRNA expressions with the RT-qPCR method and measurements of PKC α, STAT3, and mTOR protein expressions with Western blot method. **Results:** This study showed that the ethanol extract of I. cylindrica roots had an IC_{50} of 541 µg/mL (weak cytotoxic) and the IC_{50} of erlotinib was at 29 µM (moderate cytotoxic). In apoptotic test, the highest average apoptotic results were found in erlotinib group, while ethanol extract doses of 150, 300, and 600 µg/mL gave apoptotic effects on cancer cells higher than negative control group. From RT-qPCR, found that *I.cylindrica* significantly inhibit the expression of *PKC α*, *STAT3*, and *mTOR* mRNA and from band scanning using Western Blot showed PKC α, $STAT_3$, and mTOR protein expressions in Imperata root ethanol extract, qualitatively produced thinner bands when compared to the negative control group. **Conclusion:** This study demonstrated *I.cylindrica* root ethanol extract ability inhibiting the expression of PKC α, STAT3 and mTOR genes and proteins in the A549 lung cancer cell line, which can be taken into consideration as a complementary therapy in treatment of lung cancer.

Keywords: A549, cytotoxic, Imperata cylindrica, mTOR, PKC α, STAT3

INTRODUCTION

Lung cancer is the highest cause of death in the United States and worldwide, accounts for approximately 12,4% of cancer cases, the leading cause of death from cancer in men in 2008, and the second leading cause of death in women[1,2]. Epidermal Growth Factor Receptor (EGFR) biomarker was found to be over-expressed in 62% of non-small cell lung carcinoma (NSCLC) cases and resulted poor prognosis. Approximately 10% of lung adenocarcinoma patients in the United States and East Asia have lung cancer associated with EGFR mutations[3]. When an extracellular ligand binds to EGFR, it triggers homo/heterodimerization of the receptor which results in a phosphorylation process in the cytoplasmic tyrosine kinase and activates various intracellular downstream pathways, including the phosphatidyl inositol 3-kinase (PI3K)/AKT/mTOR pathway, the RAS/RAF/mitogen activated protein kinase (MAPK) pathway, the Janus kinase-STATs pathway, as well as the PLCɤ-PKC pathway which will result in cell proliferation, metastasis, and prevent apoptosis[4,5].

Protein kinase C-α shows high expression in the incidence of non-small cell lung carcinoma which is higher in adenocarcinoma compared to squamous cell carcinoma. Upregulation, increased activity and tumor-inducing ability by PKCα make this type of kinase a potential prognostic marker as well as a therapeutic target in NSCLC patients[6]. Signal transducer and activator of transcription-3 (STAT3) is a critical signaling mediator in the occurrence of malignancy and is continuously active in 22-65% of NSCLC cases. STAT3 is activated by cytokines such as interleukin-6, mediated by Janus family kinase (JAK), and acts as a downstream EGFR pathway,[7] while mTOR (mammalian target of Rapamycin) is an important intracellular regulator for growth, survival, migration and invasion. cells in the event of NSCLC. Increased mTor activation is associated with poor clinical presentation in early-stage NSCLC[8].

According to the National Comprehensive Cancer Network (NCCN) in 2021, for patients who have lung cancer with non-small cell lung carcinoma, shows good sensitivity to EGFR-tyrosine kinase inhibitor therapy, such as erlotinib, gefitinib, afatinib, osimertinib and dacomitinib[9].

Imperata cylindrica or better known as Cogon grass is a medicinal plant that thrives in Indonesia and has been used for generations as a medicinal plant.[10] Imperata has many benefits as an antiinflammatory, antioxidant, antibacterial, immunomodulator, hepatoprotector, and anticancer effect.[11] Several studies have shown that *I.cylindrica* leaf extract has an anticancer effect on human oral squamous carcinoma SCC-9 cell culture[12], as an antioxidant

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and anticancer in human breast cell culture MCF-7[13], breast BT-549 cell culture and colon cancer cell culture HT-2927.[14]This study aim to investigate the potential effect of *I.cylindrica* root ethanol extract on inhibit the expression of PKCα, STAT3, and mTOR gene and protein in A549 lung cancer cell line.

MATERIAL AND METHODS

Preparation of I.cylindrica Root Ethanol Extract

Starting with the maceration method of 100 grams *Imperata cylindrica* root simplicia powder mixed with ethanol, then filtered 5 times 24 hours at 20–22°C until the macerate and precipitate would be separated, then filtered. The collected maserate was then evaporated and concentrated using a rotavapor at 50°C to produce thick ethanol extract of 16, 320 grams of alang-alang root ethanol extract.

Preparation of A549 lung cancer cell line

A549 cells (ATCC-CCL185), a human adenocarcinoma lung cancer cell line, were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, 0.2% sodium bicarbonate and 1% penicillin/streptomycin antibiotic solution (1mL/100 mL medium). Cells were then grown in 5% CO_2 at 37°C with high humidity and allowed to stand for 24 hours to form a monolayer consisting of 80–90% confluent cells. Next, the cells were trypsinized using 0.05% trypsin to remove the monolayer from the plate and grown into 96 wells for 48 hours at 37°C with a density of 1x104 cells/well for cytotoxic examination.

MTT Assay

A 5 mg/mL MTT stock solution was obtained by adding 50 mg MTT with 10 mL of sterile PBS and stored in a dark room at -20°C and rewarmed at room temperature, filtered, and then washed using dimethylsulfoxide (DMSO). The cytotoxic effect was measured using 96 wells at a cell density of 1 × 104 in 200 µL of complete culture medium. The plates were then incubated for 12–24 hours at 37°C until >80% confluent cells were formed and tested with various concentrations for 48 hours triplicate. After incubating for 48 hours, the medium was removed, washed, and rinsed using PBS, then MTT solution was added to each well with a final MTT concentration of 0.5 mg/mL, and incubated again for 4 hours at 37°C. The remaining solution is discarded and produces blue/purple formazan crystals and is read on an ELISA multiwell plate reader with a wave of 550 nm. The examination of *I.cylindrica* root ethanol extract was carried out with a serial concentration of 1000; 500; 250; 125; 62.5; 31.75; 15.625 µg/mL and on erlotinib performed serially at a concentration of 100; 50; 25; 12.5; and 6.25 µM[15].

Apopotosis Test

The apoptosis ability was detected by staining cells using annexin V and propidium iodide solution followed by analysis using flowcytometry/ fluorescence activated cell sorting (FACS). When a cell undergoes apoptosis, the inner membrane of the cell will become the outer membrane which will be detected by annexin V, while propidium iodide will color the necrotic cells. The treated cells were placed in a 6-well plate for 48 hours with a triplicate test, removed the medium, and rinsed using PBS. After that trypsin was given for 3 minutes, after the cells were released, transferred to a large tube and centrifuged for 5 minutes, rinsed with PBS, and discarded the remaining medium and liquid. The next procedure was added 100 µL annexin binding buffer and transferred to a flex tube, added annexin 5 µL/tube and propium iodide (PI) 1 µL/tube, then vortexed, incubated at 20-22°C for 15 minutes and added another 400 µL annexin binding buffer and then read on FACS apparatus.

Quantitative RT-qPCR

Quantitative RT-qPCR was used to measure the mRNA expression of genes analyzed in this study. Total RNA was isolated from the cells using the Quick-RNA™ cDNA Synthesis Kit (Bioline Reagents Ltd., UK). Afterward, quantitative RT-PCR was performed using SensiFast™ SYBR® No-ROX Kit according to the manufacturer's instructions. The polymerase activation was set at 95°C for 2 minutes, followed by 40 cycles of denaturation at 95°C for 5 seconds and annealing/extension at 60-65°C for 20 seconds. All mRNA expression was normalized with *β-aktin* mRNA expression. The primers used in this study are listed in Table 1.

Antibody preparations

This study used two forms of protein, wild type-form and phosphorilated-form from Sigma Aldrich manufacture, with catalogue number Anti-STAT3 antibody (SAB4502873), Anti-mTOR antibody (SAB4501040), Anti-PKC alpha antibody (SAB4502354), Anti-phospho-STAT3 (ZRB1006-4X25UL), Anti-phospho-mTor (SAB4504476), Anti-phospho-PKC alpha (SAB4504057), with dilution 1:500~1:1000.

Protein detection with Western Blot

Protein detection in this study was analyzed with Westen Blot conventional methods which use color-based visualization methods such as chromogenic substrates that produce color on the membrane. Prepare materials with soaking gel in TBS-Tween 20, and membrane in transfer buffer, continue with transfer proteins by stacking filter paper, membrane, gel, and paper. Transfer at 100mA (30 min), 100V (60 min), 200V (30 min), then checking transfer by using a marker (Page Ruler®) to confirm. Block membrane using 5% skim milk in TBS (overnight, 4°C) and then wash with TBS-Tween 0.05% (3x, 5 min). For primary antibody, incubate (1:250 in TBS-BSA, 2 hours), wash with TBS-Tween 0.05% (3x, 10 min). For secondary antibody, incubate (1:250 in TBS, 2 hours, 20-22°C), wash with TBS-Tween 0.05% (3x, 5 min). Last step is to detect by adding TMB substrate (20 min), stop with water, dry, and scan.

Statistical analysis

Statistical analysis in this study was performed using SPSS ver.27 based on one-way ANOVA followed by Bonferroni post hoc test for multiple comparisons The results were expressed as mean ± standard deviation (SD). Differences between groups were considered statistically significant at $p<0.05$. With sample size less than 30 samples, we conducted normality test using the Shapiro-Wilk test. The normal sample distribution were analyze with the ANOVA test. However, an abnormal sample distribution will continued with the Kruskal-Wallis test.

Table 1. Primer sequences for RT-qPCR analysis

No.	Primer name	Sequences
1	PKCα-Fw	5' GGGACGAGGAAGGAAACATGGAAC 3'
2	PKCα-Rv	5' AACTCCCCTTTCCCAACACCATGA 3'
3	$STAT_3$-Fw	5′-CATCATGGGCTTTATCAGTAAGGA-3′
4	$STAT_3$-Rv	5′-GTCAATGGTATTGCTGCAGGTCGT-3′
5	mTOR-Fw	5'- CGC TGT CAT CCC TTT ATC G-3'
6	mTOR-Rv	5'- ATG CTC AAA CAC CTC CAC C-3'
7	β-actin-Fw	5'- ACC GAG CGC GGC TAC AG- 3'
8	β-actin-Rv	5'- CTT AAT GTC ACG CAC GAT TTC C-3'

RESULTS

Mild cytotoxic effect of *I.cylindrica* Root Ethanol Extract and Moderate cytotoxic effect of Erlotinib on A549 Lung Cancer Cell Lines

The cytotoxic test was carried out using the MTT method to determine the anticancer potential of the ethanol extract of alang-alang roots and the active compound erlotinib as a standard drug against the A549 lung cancer cell line. The result of the MTT test is the concentration required for the test compound to inhibit the growth of 50% of the cancer cell culture population (IC_{50}). The IC_{50} value was obtained from the results using the SigmaPlot ver.14 software by entering the concentration and cell viability.

Tests on the ethanol extract of alang-alang roots were carried out with a serial concentration of 1,000; 500; 250; 125; 62.5; 31.75; 15.625 µg/mL and on erlotinib performed serially at a concentration of 100; 50; 25; 12.5; and 6.25 µM. Figure 1 shown that the ethanol extract of alang-alang roots has an IC_{50} of 541 µg/mL which is in the category of having a weak cytotoxic effect and Figure 2 shown the IC_{50} of the erlotinib compound is at 29 µM, which is in the category of having a moderate toxicity effect.

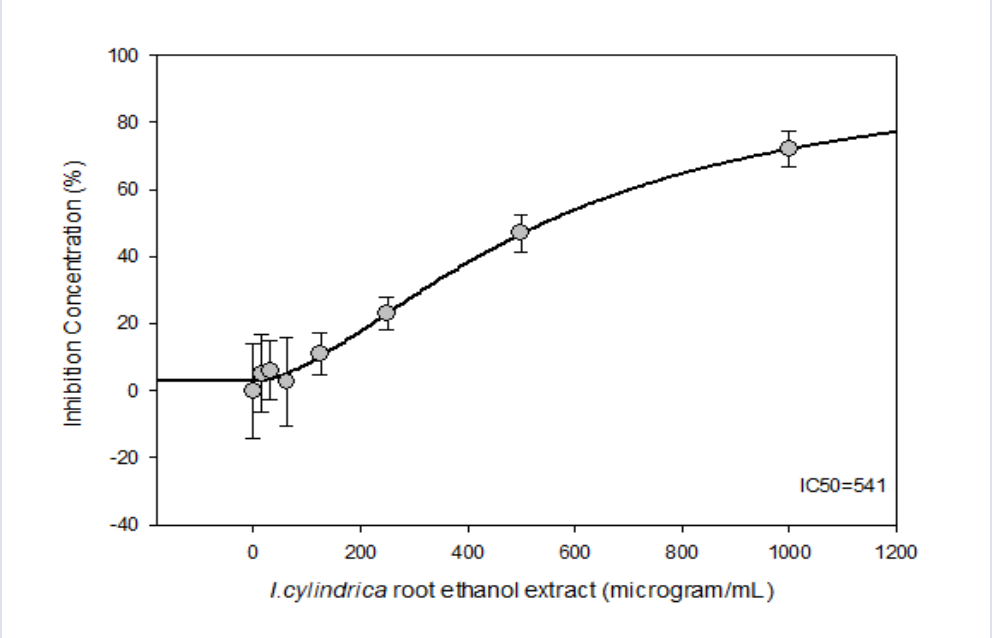


Figure 1. Inhibition Concentration (IC_{50}) Standard Curves of *Imperata cylindrical* Root Ethanol Extract in A549 Lung Cancer Cell Lines

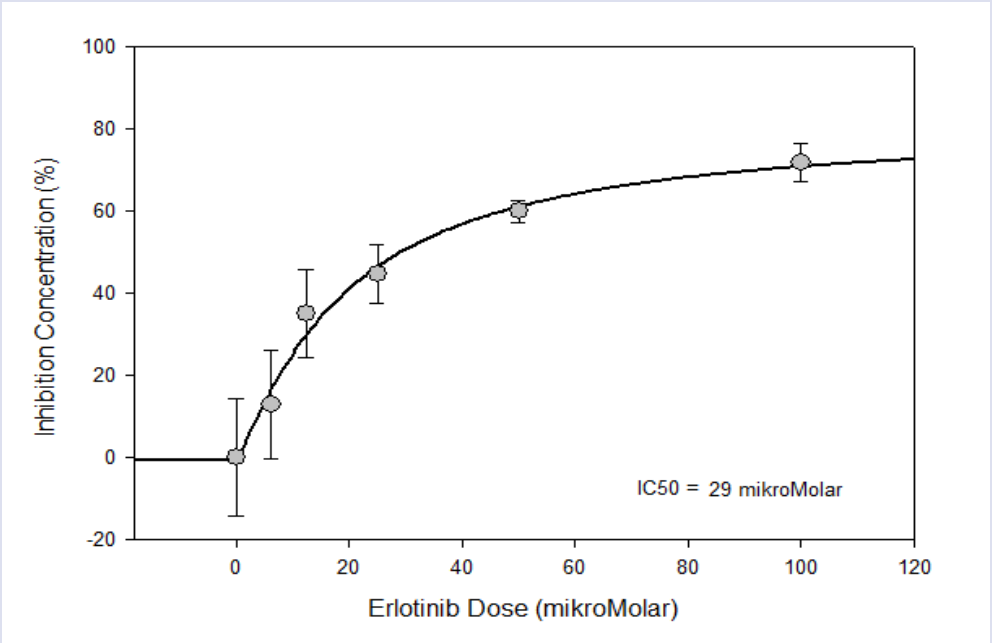
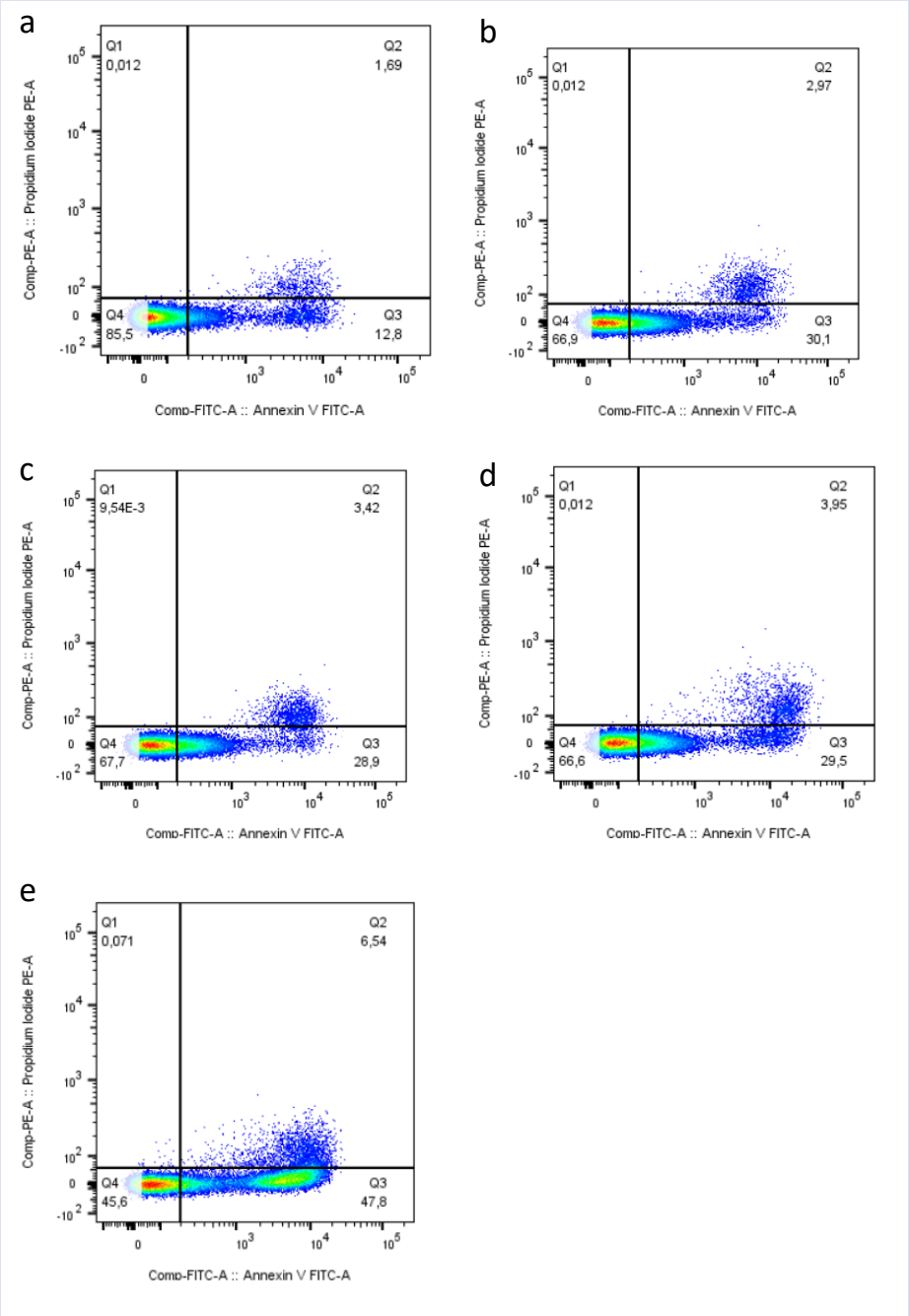


Figure 2. Inhibiton Concentration (IC_{50}) Standard Curves of Erlotinib in A549 Lung Cancer Cell Lines.

I.cylindrica induces Apoptosis on A549 Lung Cancer Cell Lines

Examination of apoptosis in this study used the flowcytometry method with fluorescence activated cell sorting (FACS-BD) on the A549 lung cancer cell line with measurement after 48 hours post-treatment with doses based on cytotoxic tests obtained IC_{50} EAA was 541 µg/mL. To facilitate calculations during dissolution, the doses were increased to 600 µg/mL, 300 µg/mL (1/2 IC_{50}), and 150 µg/mL (1/4 IC_{50}) ethanol extract of Imperata roots, and erlotinib IC50 as a positive control. Each area shows the percentage of living cancer cells (Q4), early phase of apoptosis (Q3), late phase of apoptosis (Q2), and area of necrosis (Q1) in each group both in the negative control group, positive control, and group given extract at doses of 150, 300, and 600 µg/mL can be seen in detail in Figure 3. The data shows that the apoptotic area is the result of the sum of the early apoptotic (Q3) and late apoptotic (Q2) areas, so that the total apoptotic results will be obtained in Table 2 below. Table 2 shows the mean and standard deviation of the five treatment groups. Shapiro Wilk's normality test showed that the data were normally distributed so that the analysis of apoptosis between groups used the One Way ANOVA test. The results of the One Way ANOVA test showed a significant difference in the percentage of apoptosis between groups ($p < 0.01$), therefore it was continued with the post-hoc test with Bonferroni in Figure 4.



Figures 3. Apoptosis result with Flowcytometri FACS-BD in negative control (a), *I.cylindrica* ethanol extract 150 µg/mL (b), *I.cylindrica* ethanol extract 300 µg/mL (c), *I.cylindrica* ethanol extract 600 µg/mL (d), and Erlotinib IC_{50} (e)

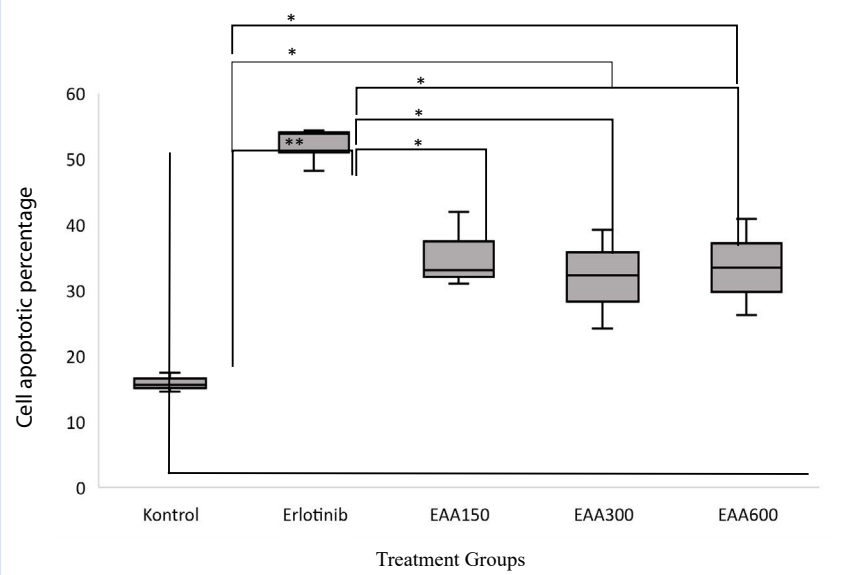


Figure 4. Apoptosis percentage comparism between groups.

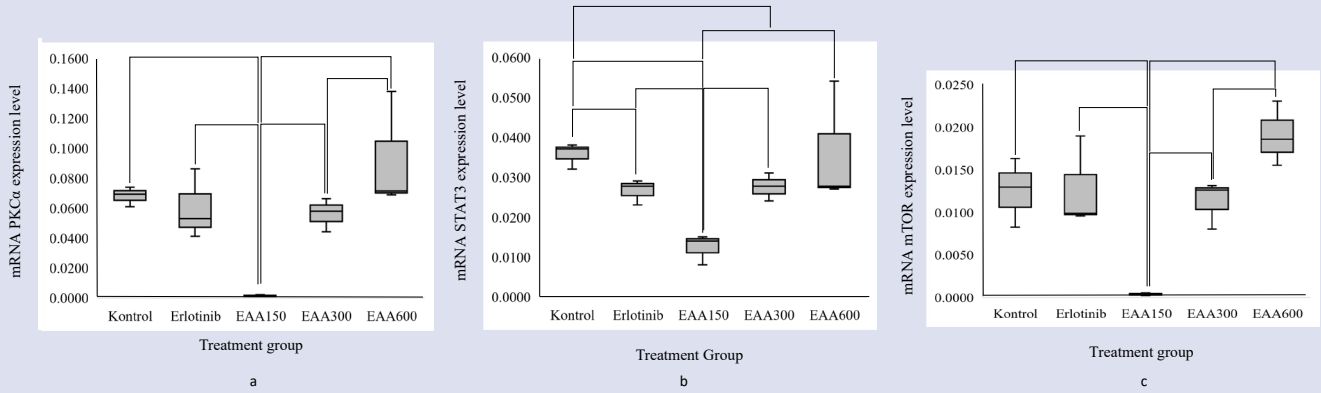


Figure 5. *PKCα* mRNA expression using RT-qPCR after 48h post treatment of Imperata cylindrica root ethanol extract (a), *STAT3* mRNA expression post treatment (b), *mTOR* mRNA expression post treatment (c).

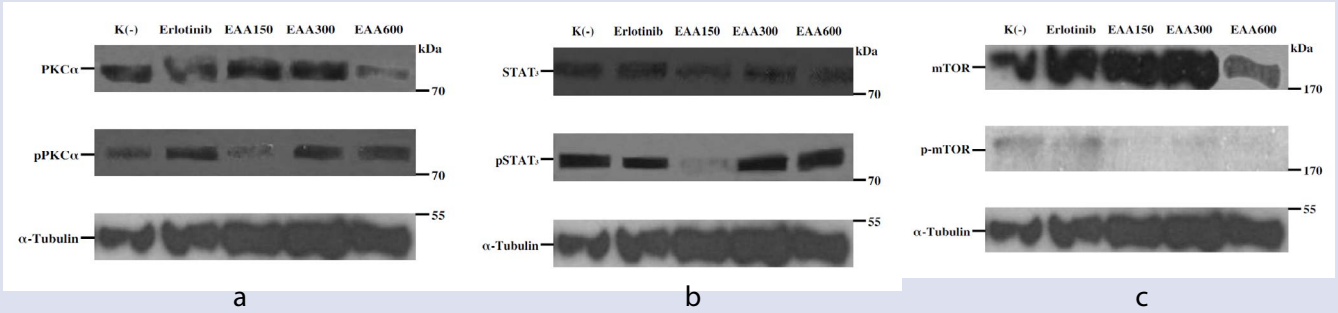


Figure 6. PKCα and pPKCα expression using Western Blot after 48h post treatment of *Imperata cylindrica* root ethanol extract (a), $STAT_3$ and $pSTAT_3$ expression post treatment (b), *mTOR* and p-mTOR expression post treatment.

I.cylindrica Root Ethanol Extract Inhibits *PKCα, STAT*$_3$ and *mTOR* mRNA expressions

In this study the measurement were performed using the Rotor-Gene Q Series Software. The data were then analyzed using the relative quantification method of comparative threshold cycle analysis or comparison of delta delta threshold (ΔΔCT or DDCT). This method compares the target with the selected reference value, namely the expression level of the housekeeping gene that is suitable in this study using the β-actin gene. In the treatment group given reed

Table 2. Total apoptosis area in Q3 and Q2 between groups

Group Intervention	Number of group	Mean±SD	p-value
Negative control	3	15,91±1,45	
EEI 150 µg/mL	3	35,35±5,78	
EEI 300 µg/mL	3	31,91±7,54	<0,01
EEI 600 µg/mL	3	33,51±7,33	
Erlotinib IC_{50}	3	52,12±3,40	

Abbreviations: EEI**:** ethanol extract of *Imperata cylindrica*

extract doses of 150 and 300 µg/mL there was lower expression of the PKCα gene compared to the negative control group, but it was seen to be higher at a dose of 600 µg/mL. The positive control group with erlotinib administration also showed lower expression of the PKC α gene compared to the control group without treatment. The abnormal distribution of data in the Kruskal-Wallis test showed significant differences in gene expression between treatment groups, so it was continued with a post-hoc test using the Mann-Whitney, the results of which are summarized in Figure 5a. In the treatment group given reed extract at doses of 150, 300 and 600 µg/mL (n=3 samples for each group), there was lower STAT3 gene expression when compared to the negative control gro up. The erlotinib group also appeared to be lower when compared to the control group without treatment. The abnormal distribution of the data after the Kruskal-Wallis test was carried out on the p value resulted in a p value <0.05 which indicated a significant difference in STAT3 gene expression so that the Mann Whitney post-hoc test was carried out and summarized in Figure 5b. The expression level of the mTOR gene mRNA in Figure 5c shows that the treatment group that was given reed extract at doses of 150 and 300 µg/mL decreased when compared to the negative control group, but there was an increase in expression at the dose of 600 µg/mL reed extract. The positive control group with erlotinib administration appeared to be slightly higher than the control group without treatment. The Kruskall Wallis test showed significant differences in mTOR gene expression between treatment groups (p value(<0.05) so it was continued with Mann Whitney post-hoc.

I.cylindrica Root Ethanol Extract Inhibits PKCα, $STAT_3$ and mTOR protein expressions

In this study, two forms of protein expression were examined, namely in the wild-type form and in the phosphorylated form. The phosphorylated form of protein is the result of a post-translational modification of a protein that is reversible in the form of amino acid residues that undergo phosphorylation by protein kinases with the addition of a phosphate group to the covalent bond of the protein. The immunoblot results in Figure 6 shows the expression of PKCα and pPKCα proteins in the culture of the A549 lung cancer cell line. The amount of protein in the whole lysate cell (WLC) was 15 µg and α-tubulin was used as a protein loading control. The molecular weight of PKCα protein is 77 kDa and α-tubulin is 52 kDa. Exposure time for PKCα protein scanning was read in the 10^{th} minute and pPKCα in the 1^{st} minute. The figure shows that the thickness of the PKCα band decreases with increasing doses of Imperata root ethanol extract and the thinnest band is produced at a dose of Imperata ethanol extract of 600 µg/mL. In pPKCα expression, the thickness of the band produced was the thinnest in the administration of 150 µg/mL dose of Imperata root ethanol extract.

Figure 6 also demonstrated the expression of $STAT_3$ and $pSTAT_3$ proteins with molecular weight of STAT3 is 87 kDa. Exposure times for scanning $STAT_3$ and $pSTAT_3$ proteins were read at 10 minutes. It was found that the thickness of the $STAT_3$ band did not show any difference in band thickness between the control group and the treatment group, whereas in pSTAT3 expression, the thickness of the band produced was the thinnest in the administration of Imperata root ethanol extract at a dose of 150 µg/mL.

Meanwhile, the molecular weight of the mTOR protein is 280 kDa. Exposure time for protein scanning mTOR was read at 60 minutes and p-mTOR was read at 10 minutes. From Figure 6 was found the thickness of the mTOR band has the thinnest band thickness at the dose of Imperata extract 600 µg/mL, whereas in the expression of p-mTOR, the thickness of the band produced in the treatment group of Imperata root ethanol extract at doses of 150, 300, and 600 µg/mL thinner than the band produced by the negative control group.

DISCUSSION

The objective of this study was to elaborate the potential effect of *I.cylindrica* root ethanol extract on inhibit the expression of gene and protein of PKCα, STAT3 and mTOR in the A549 lung cancer cell line. Our data showed that *I.cylindrica* root ethanol extract had an IC_{50} of 541 µg/mL which was in the category of weak cytotoxic effect, while erlotinib had IC_{50} 29 µM. This result almost similar from previous research which found that the IC_{50} value for erlotinib in the A549 cell line was at 23 µM–25 µM[16,17]. The weak cytotoxic effect on the ethanol extract of Imperata root might be caused by a weaker form of ethanol extract in attracting active compounds contained such as phenolic compounds, flavonoids, and alkaloids in a natural product compared to methanol. The solvent used in the extraction process has the effect of attracting different active compounds so it will affect the amount and ability of the active compounds contained therein[18]. Several chemical compounds in *I.cylindrica* such as polyphenols, flavonoids played an important roles in contributing cytotoxic effect on A549 lung cancer cell line[19,20].

In this study the apoptosis test used the flowcytometry method with fluorescence activated cell sorting tool on the A549 lung cancer cell line with readings after 48 hours post treatment at doses of 150, 300, and 600 µg/mL of ethanol extract of reed roots as well as IC_{50} erlotinib as a positive control. Based on these results it can be concluded that the apoptotic ability of cancer cells in the administration of Imperata ethanol extract is still below erlotinib as standard therapy. Erlotinib gave the highest results of inducing apoptosis of cancer cells in accordance with previous studies which stated that erlotinib could induce apoptosis of A549 cells through the activation of the ROS-dependent Janus kinase (JAK) pathway[17]. Erlotinib as a tyrosine kinase inhibitor has the ability to inhibit various signaling pathways under its regulation, including the PI3K/Akt/mTOR pathway which has a major role in the induction of apoptosis as well as the Janus kinase (JAK)/STAT3 and PLCɣ-PKCα pathways which have a major role in inhibiting the processes of transcription, protein synthesis, and cancer cell proliferation[21,22,23].

Based on the pathogenesis of lung cancer, PKCα expression was found to be increased in non-small cell lung carcinoma and higher in adenocarcinoma than in squamous cell carcinoma. Lung cancer cell lines H1355, H157, H1155, H1703, and A549 showed increased PKCα levels compared to normal human bronchial epithelial cells. In addition, increased levels of PKCα were also found in the NSCLC cell lines types PC-9, PC-14, and RERF-LC-MS[24,25]. Upregulation, increased activity and ability to induce tumors by PKCα make this type of kinase a marker potential prognostic as well as a therapeutic target in lung cancer patients[6]. Likewise, the continuous activation of the $STAT_3$ gene will strengthen the process of cell proliferation, metastasis, and angiogenesis in various types of cancer cells including NSCLC,[26] it also prevents apoptosis so that cancer cells become more resistant to cytotoxic drugs, whereas in the mTOR pathway, mTOR1 plays a role. in the regulation of protein translation, synthesis of nucleotides and lipids through 4E-BP1, S6K1, and their effectors below, while mTOR2

regulates SGK (serum glucose kinase) and PKC (protein kinase C) to increase cell survival, cytoskeleton reorganization, and cell migration[27].

The ability of the ethanol extract of the alang-alang roots can be seen in Figure 5a which shows the expression of the PKCα gene which was lower in the administration of the ethanol extract of the alang-alang roots at doses of 150 and 300 µg/mL compared to the negative control group but it appeared to increase slightly in dose of 600 µg/mL. This is based on the dose-response curve in the pharmacodynamic process of the drug, where there is a concentration or maximum amount of drug that can bind to the site of action or what is commonly called maximal efficacy, so that the drug potency does not always give rise to a linear picture and gives rise to a slope on the dose-response curve[28].

The proteins analyzed in this study consisted of 2 forms, namely the natural form or which had not undergone a wild-type change and the protein in the form which had undergone post-translational modification, namely phosphorylated protein. Protein phosphorylation is a reversible cellular mechanism elicited by various protein kinases and consists of adding a phosphate group to the polar moiety of an amino acid. The addition of this phosphate group causes the protein which was originally apolar hydrophobic to change to polar hydrophobic which will give a structural change when interacting with other molecules. Phosphorylated amino acids can bind to molecules that can interact with other proteins to form or attach to various protein complexes. Thus, it has an important role for coordination and carrying out important functions such as regulation of cell metabolism, proliferation, apoptosis, inflammation, and various other physiological processes[29,30]. The results of analysis of protein expression in both the wild-type and phosphorylated forms resulted in decreased expression of PKCα, STAT3, and mTOR proteins at various treatment doses of Imperata root ethanol extract. Although protein expression is not always linear with the result of gene expression that is formed, protein in its active phosphorylated form is the final form of a series of protein formation which has the function of regulation, proliferation, and apoptosis in the next process.

The results of a non-linear relationship between dose and effect in this study could be due the resulting gene expression does not always result in linear protein expression,[31] or due to the interaction between the chemical compounds contained in the ethanol extract of Alang-alang roots. Theoretically in medicinal plants, apart from the active substance as the main component which has the most effect, there are still other side compounds, which might affect the expected response. In the ethanol extract there are secondary metabolites such as: glycosides, saponins, alkaloids and others. These compounds will affect the results of the response. At small concentrations, these side compounds are still present in small amounts and have not affected or interfered with the expected results. At higher concentrations, the effects of these side compounds become larger in comparison so that they begin to have an effect and interfere with the response. This occurs at high concentrations, side compounds will interfere with the expected response results.[32]

The following limitation of this research are that cytotoxic effect in this study was only carried out through the apoptosis induction pathway without analizing cell cycle pathway. This research also use crude extract rather than purified compounds considering it could be more applicable in daily use in society.

CONCLUSION

This study reveals the cytotoxic effect of *Imperata cylindrica* root ethanol extract to inhibit the expression of PKCα, STAT3 and mTOR genes and proteins in the A549 lung cancer cell line, suggesting the potential of this plant as a complementary therapy in treatment of lung cancer. Although in some higher dose show contradictory effects, it is based on the dose-response curve in the pharmacodynamic process of the drug, where there is a concentration or maximum amount of drug that can bind to the site of action or what is commonly called maximal efficacy, so that the drug potency does not always give rise to a linear picture and gives rise to a slope on the dose-response curve.

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