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The PI3KCA and AKT Inhibitory Activities of *Litsea Cubeba* Lour. Fruits and Heartwoods Towards Hela Cells

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Keywords: Litsea cubeba Lour; Fruits; Heartwoods; PI3KCA; Akt

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Abstract

AIM: To investigated the activities of chloroform fractions at pH 7 of *Litsea cubeba* Lour. Fruits and heartwoods (CF-7F and CF-7H) in decrease expression of PI3KCA, Akt-1 and Akt-2 genes towards cervical cancer cell culture (HeLa) experiments in vitro.

MATERIAL AND METHODS: CF-7F and CF-7H (12.5 and 25 µg/mL) were tested for its potential inhibition on gene expression of *PI3KCA, Akt-1* and *Akt-2* genes by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) method.

RESULT: CF-7F and CF-7H were showed the activity to reduce the expression of *PI3KCA, Akt-1* and *Akt-2* genes.

CONCLUSION: Our results suggest that CF-7F and CF-7H significantly inhibit the expression of *PI3KCA, Akt-1* and *Akt-2* genes.

Introduction

Cancer is one of the high incidences of dangerous diseases in human and presently there is a considerable number of new anticancer agents from natural products [1]. According to WHO data, cancer is one of the leading causes of death worldwide especially cervical cancer [2]. Cervical cancer therapy with chemotherapeutic agents is the limited cause of drug resistance, and toxic side effect on normal tissue leads to some effects such as immunosuppression and cardiotoxicity [3], [4]. Phosphatidylinositol 3-kinase/AKT/mTOR pathway plays an important role in proliferation, migration and survival. Activation of the phosphatidylinositol 3-kinase/AKT/mTOR pathway is attained through the mutation in the p110α subunit of the *PI3KCA* gene which leads to the development of cancer.

Attarasa (*Litsea cubeba* (Lour.) is a plant from Lauraceae family which contain many essential oils which used as antidepressants, antiinflammation, antioxidant, pesticide, antimicrobial, anticancer on breast cancer and neuropharmacology. The methanol extract from attarasa fruits showed to be active on HeLa cell lines which cause apoptosis through activation of caspase 3/7 [5], [6]. There are more than forty isoquinoline alkaloids that contained in *Litsea* genus which are active as antibacterial agents against *Staphylococcus aureus* [7]. The heartwoods of *Litsea cubeba* contained a high level of phenolic and flavonoid and found to be active as an antioxidant and

has anti-breast cancer activity which causes cell cycle inhibition. Alkaloids compound which isolated from heartwood has antioxidant activity with DPPH and ABTS methods [8], [9], [10].

This study aimed to assess PI3KCA, Akt-1 and Akt-2 inhibition activity of *Litsea cubeba* Lour. Heartwoods on HeLa cells.

Material and Methods

Fresh heartwoods and fruits of *Litsea cubeba* Lour. Was collected from Balige subdistrict, Sumatera Utara province, Indonesia. The air-dried and powdered heartwoods of *Litsea cubeba* (Lour.) (1 kg) were repeatedly macerated with ethanol 96% (3 x 3 d, 7.5 L), The filtrate was evaporated to give a viscous extract. Viscous extract was fractionated with n-hexane and continue with chloroform at pH 3,7 and 9 [10], [11], [12].

Cells were seeded at a concentration of 5 × 10^5 cells mL^{-1} in 6-well plates and cultured for 24 h in RPMI 1640. The cells were incubated without treatment (Control cell) and treatment for 24 h. The treatment group is chloroform fractions of fruit and heartwood *Litsea cubeba* Lour. at concentrations 12.5 and 25 µg mL^{-1}. Cells were harvested and placed in microtube [9].

The gene expression of PI3KCA, Akt-1 and Akt-2 were determined by RT-PCR. Total RNA from the control cell and treatment groups were extracted using the Total RNA Mini Kit (Geneaid) according to the manufacturer's protocol. The oligonucleotide primers for PI3KCA, Akt-1, Akt-2 and beta-actin were shown in Table 1.

Table 1: Mouse oligonucleotide primers sequences used for RT-PCR (5-3') and Annealing temperature

Gene		Primer Sequences	Size (bp)	Temp (°C)
AKT-1	F	5'-ATGAGCGACGTGGCTATTGTGAAT-3'	330	58
	R	5'-GAGGCCGTCAGCCACAGTCTGGATG-3'		
AKT-2	F	5'-ATGAATGAGGTGTCTGTCATCAAAGAAGGC-3'	315	55
	R	5'-TGCTTGAGGCTGTTGGCGACC-3'		
PI3KCA	F	5'-GGACAATCGCCAATTCAG-3'	300	53.5
	R	5'-TGGTGGTGCTTTGATCTG-3'		
β-actin	F	5'-GCTCCTCCTGAGCGCAAGT-3'	105	58
	R	5'-TCGTCATACTCCTGCTTGCTGAT-3'		

PCR consisted of 35 amplification cycles, and each cycle was carried out for 30 s at 95°C, 1 min at annealing temperature (58°C for AKT-1 and beta-actin), (55°C for AKT-2), (45 s at 95°C, 1 min at annealing temperature 53.5°C for PI3KCA.) and 1 min at 72°C in a thermal cycler (ProFlex™ 3 x 32-well PCR System, Applied Biosystems). The beta-actin housekeeping gene was used as an internal control to standardise the relative expression levels for all biomarkers. PCR products were separated electrophoretically on a 2% agarose and fluorosafe (Smobio) with Tris-Borate-EDTA (Vivantis), TBE 0,5x. The stained gel was visualised by using Gel-Doc Quantity One software (Syngene) [13].

Triplicate experiments were performed throughout this study. All data were presented as the mean ± Standard Error Mean (SEM), which were analysed using the SPSS 22 software. The significant difference between control and treated groups were analysed by the paired Student's t-test ($p < 0.05$).

Results

Effects extract *Litsea cubeba* Lour. Fruits and Heartwoods on the genes expression (AKT-1, AKT-2 dan PI3KCA).

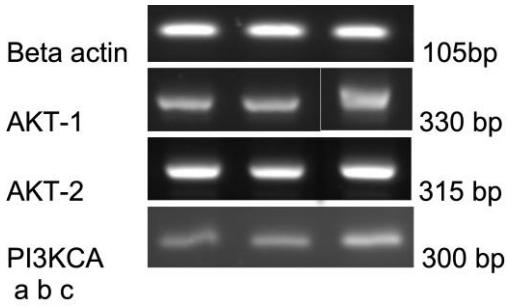

Figure 1: Effects extract Litsea cubeba Lour. Fruits and Heartwoods on the gene expression in Hella cell. The total RNAs were isolated, and RT-PCR was performed using the indicated primers in Materials and Methods. (a), CF-7H (b), CF-7F (c), Control cells. RT-PCR, reverse transcription-PCR; Bp, Base Pair

Furthermore, we tested whether CF-7H and CF-7F have anticancer activity in inhibition the expression of PI3KCA and AKT genes. As shown in Figure 1 and Table 2, treatment with CF-7H and CF-7F (25 and 12.5 µg mL^{-1}) inhibited the gene expression of PI3KCA, Akt-1 and Akt-2.

Table 2: The score of genes expression after treatment with CF-7H and CF-7F

No	Gene	CF-7H	CF-7F	Control
1	Beta actin	1.04 ± 0.01	1.03 ± 0.01	1.00 ± 0.00
2	*AKT-1*	0.83 ± 0.02	0.81 ± 0.02	1.00 ± 0.00
3	*AKT-2*	0.84 ± 0.03	0.72 ± 0.01	1.00 ± 0.00
4	*PI3KCA*	0.77 ± 0.01	0.63 ± 0.02	1.00 ± 0.00

Discussion

In this present study, we demonstrated that *Litsea cubeba* Lour. Heartwoods and fruits exhibited anticancer activity on decreasing the expression of PI3KCA, Akt-1 and Akt-2. PIK3CA gene expression is associated with tumour aggressiveness in breast

cancer pattern interfering that PIK3CA deregulation may have important biologic and cellular consequences in oncogenic development pathway from transforming through progression. Akt is well-known major regulatory signalling cascade that control cell proliferation, metabolism and survival of cancer cells. Therefore, several inhibitors, such as everolimus, have been developed and used for treatment to induce apoptosis in cancer cells [14], [15]. Our results indicated that the expression levels of various genes including PI3KCA, Akt-1 and Akt-2 were downregulated as evident from the RT-PCR assay. These results indicate the potential of *Litsea cubeba* Lour. Heartwoods and fruits to inhibit cancer cell growth via inhibition of the PI3K/Akt pathway.

Based on the results, we summarised that *Litsea cubeba* Lour. Heartwoods and fruits significantly ameliorated the gene expression of PI3KCA, Akt-1 and Akt-2 towards HeLa cells.

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