The Activity of Alkaloid Fraction of *Litsea cubeba* Lour. Heartwoods on Down Regulation Cyclin D1 Expression against HeLa Cell Line

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Keywords : Anticancer, *Litsea Cubeba* Lour. heartwood, Alkaloid, Cervic Cancer.

Abstract : Objective: This study was to determined the activity of *Litsea cubeba* Lour. heartwood alkaloid fraction on down regulation cyclin D1 expression towards HeLa cell line. Methods: The heartwood powder of *Litsea cubeba* Lour. was macerated by ethanol 96% and fractionated with n-hexane and chloroform at pH 3,7 and 9. Antiproliferation study was using MTT assay and cyclin D1 expression analysis was using flow cytometry method from chloroform fraction at pH 7. Results: The IC_{50} of chloroform fractions at pH 7 was 52.46 ± 0.34 µg/mL. The fractions of chloroform at pH 7 in concentration of 25 and 10 µg/mL were as antiproliferation assay with viable cells at 53.57 ± 0.28% and 68.43 ± 0.48% respectively after 72 h incubation and decreased cyclin D1 expression (69.10 and 67.63%). Conclusion: The results reveal that *Litsea cubeba* Lour. heartwood alkaloid fractions provide effective as anticancer with mechanism through down regulation of cyclin D1. Our further study is to isolate alkaloid compounds and assess its moleculer mechanism.

1 INTRODUCTION

Cancer is a serious condition in human and nowadays there is a high amount of novel anti-cancer drugs out of natural subtances (Sharma, 2011). Based on World Health Organization report, cancer is one of the high lead of death global especially cervical cancer (Berington and Lall 2012). Cervical cancer treatment with chemotherapy drugs has limited because of resistance problem and toxic impact on normal tissue which leads to cardiotoxicity and cause of immunosuppression (Tyagi, 2004) and (Jemal, 2010)

Attarasa or *Litsea cubeba* (Lour,) is a plant which contain volatile oils used as antimicrobial, antideppresant, antioxidant, anticancer on breast cancer, pesticide, antiinflammation and neuro pharmacology. Methanol extract of attrasa fruit of indicate active on HeLa cell lines (cervical cancer) promote apoptosis with induction of caspase 3 and 7 (Piyapat, 2013), (Trisonthi, 2014). *Litsea* genus contain over than 40 isoquinoline alkaloids in which are active as antibacterial activity (Feng, 2009). The *Litsea cubeba* heartwood is conceived superior amount of flavonoid, phenolic and active as antioxidant and has anti breast cancer effect through cell cylce inhibition. Alkaloids which isolated from *Litsea cubeba* heartwood have radical scavenging activity with ABTS and DPPH (Dalimunthe, 2018), (Dalimunthe, 2016) and (Dalimunthe, 2017) The aim of this reseach was to evaluate antiproliferative activity and cyclin D1xpression of alkaloid fraction of Attarasa heartwood towards HeLa cells

2 MATERIAL AND METHOD

2.1 Extraction and Fractination

Extract and fractions were obtained as previously describe (Dalimunthe, 2018), (Hasibuan, 2016), (Rosidah, 2018) dan (Satria, 2015).

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2.2 Cytotoxic Activity

The inoculums seeded on a 96 well plate (1 x 10^4 cells/mL) and incubated at 37^0 C for 24 hours. The medium was discharged and treated with CF-7 and incubation for 24 h, the further procedure were followed as previously describe (Nurrochmad, 2011), (Satria, 2017) and (Hasibuan, 2015).

2.3 Antiproliferative Activity

Alkaloid fraction was submitted for antiproliferative activity. In that way, HeLa cell line (2.5 x 10^3cells/mL) was grown in RPMI complete medium. After 24; 48 and 72 h treatment, MTT assay was performed and cell viability was counted to calculate the antiproliferative activity (Zihlif, 2013), (Harahap, 2018).

2.4 Cyclin D1 Expression

HeLa cells (750.000 cells/well) were seeded into 6-well plate and incubated for 24 h in incubator CO2 5% . for the treatment , harvested and analysis of cells with flow cytometer were followed procedure from (Satria, 2017) with added cylin D1 antibody which labelled with FITC.

3 RESULTS

3.1 Inhibitory Concentration 50% (IC_{50})

The result of treatment CF-7 can be seen in the Table 1.

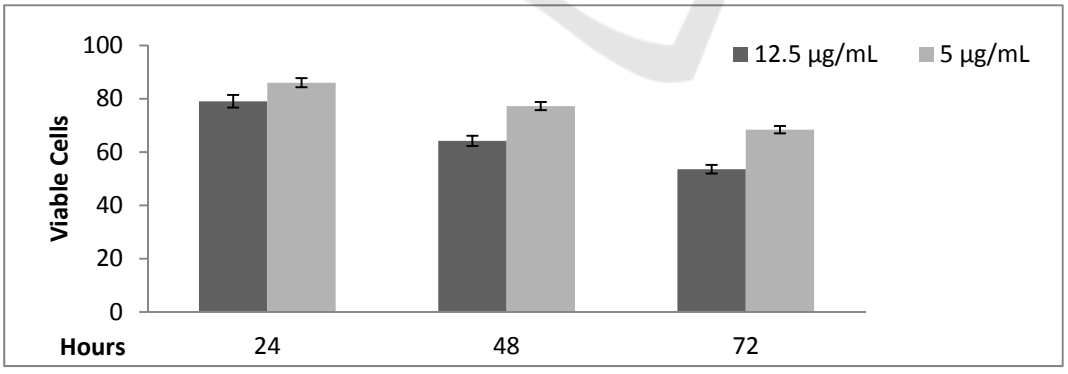
Table 1. IC_{50} value of alkaloid fraction at pH 7 of *Litsea cubeba* heartwood with MTT assay

Treatment	IC_{50} (µg/mL)(n=3)
Chloroform Fraction pH 7 (CF-7)	25.88 ± 0.16

3.2 Antiproliferative Activity

To evaluate the effect of CF-7 to decrease the number of cells by inhibiting cell proliferation. The percentage of viable cells after treatment with alkaloid fraction in 25 µg/mL and 10 µg/mL and incubation for 24, 48 and 72 h (79.08 ± 0.45 and 86.05 ± 0.31; 64.24 ± 0.41 and 77.27 ± 0.28; 53.57 ± 0.28 and 68.43 ± 0.48) respectively showed the inhibition effect of alkaloid fraction towards proliferation of HeLa cells. The effect of CF-7 is given in Figure 2

Figure 2. Percentage of viable cells of HeLa cells were treated by alkaloid fraction at pH 7 for 24; 48 and 72 h.



3.3 Analysis of Cyclin D1 Expression

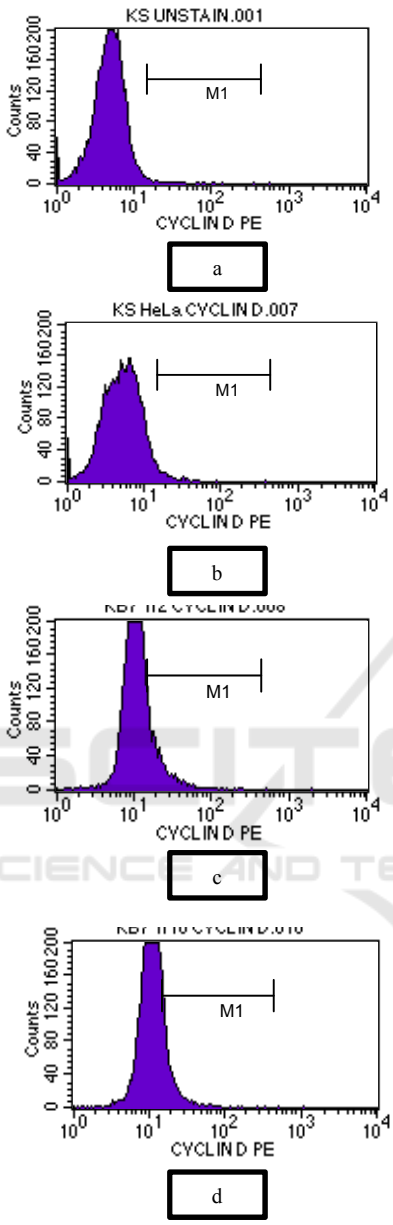


Figure 3. Analysis of cyclin D1 with flow cytometry. HeLa cells were treated by CF-7.(a) control cells unstaining; (b) control cells; (c) CF-7 25 µg/mL; (c) CF-7 10 µg/mL.

To evaluate the effect of CF-7 to decrease cyclin D1 expression, we concentrated on it for further studies using the flow ytometrymethod. The effect of CF-7 is given in Figure 3. Whiles treatment of CF-7 in 25 and 10 µg/mL caused cell accumulation in M1 area (14.60% and 13.06%) and for control cell.

4 DISCUSSION

Alkaloids have cytotoxic activity through various pathway (Sun, 2009). *Litse*a genus contain many of isoquinoline alkaloids (Feng, 2009). Alkaloids are the main compound which potentially in prevents the proliferation of cancer which inhibits proliferation of multiple cancer cell line by apoptosis stimulation and inducing cell cycle arrest at G_0/G_1 or G_2/M phases (Tang, 2009), (Burgeiro, 2011) and (Eom, 2010). Interdiction of invasion and metastasis in tumor is the one of mechanism of action of isoquinoline alkaloids in inhibits cancer growth (Ho, 2009) and (Liao, 2005). The other mechanism of quinolone alkaloid are induces DNA damage, inhibits topoisomerase enzyme, exhibit G_2/M phase arrest (Huang, 2004) and (Kans, 2004)

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