PHYTOCHEMICALS CONSTITUENT AND ANTI-PANCREATIC CANCER ACTIVITY OF ETHANOL EXTRACT OF *Litsea cubeba* Lour. FRUITS

Aminah Dalimunthe¹,²,³, Urip Harahap³, Jansen Silalahi³ and Denny Satria³

¹Department of Pharmacology Faculty of Pharmacy, Universitas Sumatera Utara, Medan, 20155, Indonesia,
²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, 20155, Indonesia
³Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, 20155, Indonesia

✉Corresponding Author: aminah@usu.ac.id

ABSTRACT

*Litsea cubeba* (Lour.) is a plant from Lauraceae family which is potential anti pancreatic cancer agent. This study evaluated phytochemicals constituent and anti pancreatic cancer activity of ethanol extract (EE) of *Litsea cubeba* Lour. fruits towards Panc-1 cell line. EE was analyzed for phytochemical constituents and tested for cytotoxicity, cell cycle inhibition, apoptosis induction and inhibition of PI3KCA, Akt-1, Akt-2, mTOR and VEGFR-2 genes expression. EE was found to contain alkaloids, flavonoids, steroids/triterpenoids, saponins, tannins and glycosides. EE of *Litsea cubeba* Lour. fruits were found to have IC₅₀ of 72.86 ± 1.03 µg/mL, caused accumulation in G₂-M phase, increased apoptosis and decreased PI3KCA, Akt-1, Akt-2, mTOR and VEGFR-2 genes expression. The results reveal that EE of *Litsea cubeba* Lour. fruits have cytotoxicity effect through cell cycle inhibition and induction apoptosis.

**Keywords:** Pancreatic Cancer, Phytochemicals Constituent, *Litsea cubeba* Lour., Ethanol Extract.

INTRODUCTION

Pancreatic cancer is one cancer which a high incidence in a developing country. Approximately 400,000 new cases occurred in 2018 and 331,000 death every year. Alteration in lifestyle and the daily diet is the most cause that affecting the number of pancreatic cancer patients. The high number of incidence and the high charge treatment for cancer patients therefore is a serious problem for looking for another source of medicine notably through traditional medicine to treat pancreatic cancer patients.¹-⁶ *Litsea cubeba* (Lour.) is a Lauraceae family plant which has contains volatile oils used as antimicrobial, anticancer on breast cancer, pesticide, antidepressants, antiinflammation, antioxidant, and neuro pharmacology. It was showed to be active on HeLa cell which causes apoptosis through the initiation of activation of caspase 3/7.⁷-⁸ Isoquinoline alkaloids in *Litsea* genus are active as antibacterial.⁹ The heartwoods contained a high concentration of flavonoid and phenolic and active as an antioxidant and inhibit breast cancer progression through cell cycle arrest. Alkaloid fractions of heartwoods and fruits can decrease PI3KCA, Akt-1 and Akt-2 gene expression. Alkaloid compounds from heartwood have an antioxidant effect with ABTS and DPPH methods.¹⁰-¹³ The purpose of our study was to determine phytochemicals and assess antipancreatic cancer activity of ethanol extract of *Litsea cubeba* Lour. fruits on Panc-1 cells.

EXPERIMENTAL

Preparation of Extract

The air-dried and powdered fruits of *Litsea cubeba* (Lour.) (1000 g) were macerated with ethanol 96% (3x3 d, 7.5 L), The filtrate was evaporated to give a viscous extract.¹⁰,¹²

Phytochemicals Constituent Analysis

Analysis of phytochemicals from EE was analyzed to determine alkaloids, flavonoids, steroids/triterpenoids, tannins, glycosides and saponins based on previous study.¹⁴-¹⁵


http://dx.doi.org/10.31788/ RJC.2021.1415609

This work is licensed under a CC BY 4.0 license
Cytotoxicity Activity

Panc-1 cell line (1x10⁴ cells) were grown in DMEM complete medium. After 24 hours incubated, then discharged and treated by EE. The further procedure was followed as previously describe.¹⁶-¹⁷

Cell Cycle Inhibition Analysis

Panc-1 cell line (1x10⁶ cells) were seeded and incubated in incubator CO₂ 5%. for the treatment, harvested and analysis of cells with flow cytometer were followed the procedure from the previous study.¹⁸

Observation of Apoptosis

Panc-1 cell line (1 x 10⁵ cells) were seeded into 24-well plate on coverslips and incubated for 24 hours. After that, the cells were treated and incubated for 24 hours. The 10μL acridine orange-ethidium bromide was added and incubated for 15 minutes and inspected under the confocal microscope.¹⁹

Expression of PI3KCA, Akt-1, Akt-2, mTOR and VEGFR-2

Panc-1 cell line (1x10⁶ cells) were seeded into 6-well plate and incubated for 24 h. After that, the cells were treated with EE 15 µg/mL and then incubated for 24 h. Isolation RNA and cDNA synthesis procedure were adopted from previous study.¹⁵,¹⁷ The gene expression of PI3KCA, Akt-1, Akt-1, mTOR and VEGFR-2 were determined by RT-PCR. The oligonucleotide primers for PI3KCA, Akt-1, Akt-1, mTOR and VEGFR-2 and beta-actin were shown in Table-1.

Table-1: Primers Sequences, Size Product and Annealing Temperature.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequences</th>
<th>Size (bp)</th>
<th>Temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGFR-2</td>
<td>F 5' - GTGTCAGAATCCCTGCGAAATGTA -3' R 5' - GAAAATGGGATTTGTAAGGAGT -3'</td>
<td>280</td>
<td>55,5</td>
</tr>
<tr>
<td>Akt-1</td>
<td>F 5' - ATGAGCCGACGCTGCTTGTGAAT -3' R 5' - GAGGGCCGTCACTGACAGCTGATGAGT -3'</td>
<td>330</td>
<td>58</td>
</tr>
<tr>
<td>Akt-2</td>
<td>F 5' - ATGAAATGAGGTTGCTGTGCTGATCAAGAAGCAGG -3' R 5' - TGCTTGGAGGCTGCTTGACC -3'</td>
<td>315</td>
<td>55</td>
</tr>
<tr>
<td>mTOR</td>
<td>F 5' - CCAATCATCGATCATTCAATCCATCC -3' R 5' - AACAAACTCATGTCCGTGCTTGACC -3'</td>
<td>315</td>
<td>55</td>
</tr>
<tr>
<td>PI3KCA</td>
<td>F 5' - GGACAATCGCCAAATTCAG -3' R 5' - TGGTTGCTGTCTTTGATGCTC -3'</td>
<td>300</td>
<td>53.5</td>
</tr>
<tr>
<td>β-actin</td>
<td>F 5' - GCTCATCCTCAGGCGCAAGT -3' R 5' - TCGTCACTCCTGCTTGATG -3'</td>
<td>105</td>
<td>58</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Phytochemicals Constituent of EE

Phytochemicals constituent analysis from EE was determined to obtain the information of the group of phytochemicals contain in EE. The result was given in Table-2. The results were showed that EE contains some phytochemicals such as flavonoids, alkaloids, saponins, tannins, glycosides and steroids/triterpenoids.

Cytotoxic Activity

The IC₅₀ value of EE on Panc-1 cells was 72.86 ± 1.03 µg/mL. The cytotoxicity estimate of a natural product is related to the content of active compounds in these plants. Flavonoids, steroids/ triterpenoids and alkaloids are estimated as active compounds.²⁰,²¹

Effect on Cell Cycle

The effect of EE is given in Fig.-1. Whereas treatment of EE 15 µg/mL caused cell accumulation at G₂/M phase (32.25%) and for control cell (23.81%).

Table-2: Phytochemicals Constituent of EE

<table>
<thead>
<tr>
<th>No</th>
<th>Chemical Compounds</th>
<th>Simplex</th>
<th>Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>No.</td>
<td>Compound</td>
<td>Presence</td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>-----------------------------------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Tannins</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Glycosides</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Steroids/Triterpenoids</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

**Apoptosis**

Figure-2 showed EE was induced apoptosis. Apoptosis Panc-1 cells were observed used confocale microscope. Panc-1 cells were treated with EE with a concentration 15 µg/mL. Apoptosis can be defined as a process of programmed cell death and this process depends on the biochemical mechanism of the cell.

**PI3KCA, Akt-1, Akt-2, mTOR and VEGFR-2 Genes Expression**

RT-PCR method was used to evaluated PI3KCA, Akt-1, Akt-2, mTOR and VEGFR-2 genes expression on Panc-1 cells after the treatment with EE 15 µg/mL. EE was showed a significant down-regulatory effect on the expression of PI3KCA (0.67±0.01); Akt-1 (0.57±0.02); Akt-2 (0.82±0.01); mTOR (0.68±0.03); and VEGFR-2 (0.85±0.02) after treatment EE at 15 µg/mL. The inhibition of EE towards PI3KCA, Akt-1, Akt-2, mTOR and VEGFR-2 gene expression on Panc-1 are given in Fig.-3.

PI3KCA, Akt-1, Akt-2 and mTOR is one of kinase protein which regulates the development of cancer through PI3K/Akt/mTOR pathway and flavonoids can reduce the expression of serin/threonine kinase, Akt, mTOR, MAPK, ERK1/2, JNK proteins. VEGFR-2 is a transmembrane receptor that plays an important role in endothelial cell development and is thought to mediate the key effect of the endothelial-specific mitogen VEGF on cell proliferation and permeability. Therefore, the majority of VEGFR-2 actions are related to angiogenesis. VEGFR-2 receptors and VEGFR-2 mRNA are largely expressed in breast cancer.
CONCLUSION

The results reveal that ethanol extract of *Litsea cubeba* Lour. fruit contains flavonoids, steroids/triterpenoids, tannins, alkaloids, saponins and glycosides compounds and effective as anticancer towards Panc-1 cell lines by several mechanisms such as cell cycle inhibition, apoptosis induction and decreases PI3KCA, Akt-1, Akt-2, mTOR and VEGFR-2 genes expression.

ACKNOWLEDGEMENT

This research was funding by Universitas Sumatera Utara through Non PNBP USU funding 2019 research grant No. 4167/UN.5.1.R/PPM/2019 (01 April 2019).

REFERENCES


