

## Short Communication

# Identification of Alkaloid Compounds in Papaya Leaves Extract (*Carica papaya* L.) with LC-MS/MS

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**Papaya (*Carica papaya* L.) contains alkaloid compounds, saponins and flavonoids. The purpose of this study was to identify the alkaloid compounds in papaya leaves extract using LC-MS/MS analysis. The carpaine identification was using Hypersild Gold column with 0.1% formic acid mixture in water and 0.1% formic acid in acetonitrile with 0.3 ml/min flow rate. ESI was used in positive ion mode with SRM (Selected Reaction Monitoring) method. The target carpaine compounds were observed at 479 m/z as precursor ions and at 240 m/z as product ions.**

**Key words:** Alkaloids, carpaine, papaya, LC-MS/MS

Papaya (*Carica papaya* L.) contains alkaloid compounds, saponins and flavonoids in its leaves, root, and bark. The phytochemical screening of simplicial powder and plants samples in wet form can identify the presence of alkaloid compounds, flavonoids, steroids, tannins, and saponins (1,2). *Carica papaya* L. is one of the plants that has the anticancer ability by increasing apoptosis and inhibiting proliferation of cells. Papaya leaves methanol extract has inhibitory activity against DNA enzyme Topoisomerase II. This is an enzyme that plays an important role in the process of replication, transcription, DNA recombination and proliferation of cancer cells (1,3). This study was aimed to observe papaya leaves extract as anticancer through inhibition of cell proliferation and apoptosis induction. It was exploratory research to observe the presence of chemical compounds contained in papaya leaves from LIPI (Indonesian Research Center) Conservation Hall Plant Garden Purwodadi Pasuruan, East Java. This research has ethical innovation established by Research Ethics Commission of Faculty of Veterinary Medicine of Airlangga University with Number: 408-KE

### *The making of papaya leaves chloroform fraction*

The papaya leaves which were slightly darker green

coloured were taken during sunny weather conditions (2). Papaya leaves were extracted using methanol solvent and adjusted to 9 pH using 5% NH<sub>4</sub>OH. The chloroform fraction obtained was then evaporated with rotavapor to obtain a chloroform fraction (2,4). The carpaine test was performed using 0.1 g in 10 mL methanol. The sonication was done for 10 minutes at 4500 rpm. The supernatant was filtered with PTFE filter 0.2 microns. The filtrate was inserted in vial bottle and 2 il injection sample volume was analyzed by LC-MS / MS.

### *Carpaine analysis with LC-MS*

Carpaine compounds were tested using LC-MS/MS equipment [UHPLC (Thermo Scientific ACCELLA type 1250)]. The column used was Hypersil Gold (50mm x 2.1mm x 1.9 im). The mobile phase A comprised 0.1% sulfuric acid diluted in distilled water, phase B consisted of 0.1% formic acid diluted in acetonitrile. A linear gradient with a speed of 300 il/min with motion phase settings was used as follows: 0-0.6 minutes 10% B, 2.5-4.0 minutes 100% B and 4 minutes 10% B. The LC injection volume was 2 il. The columns were set to 30° C and the autosampler compartment was set to 16° C. The operation of MS/MS Triple Q (quadrupole) TSQ QUANTUM ACCESS MAX mass spectrometer from Thermo Finnigan with an ionization ion ESI (Electrospray ionization) was controlled by TSQ Tune software positive mode. The quantity determination by the SRM (Selected

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Reaction Monitoring) method was set to 479 m/z as the precursor ion and at 240 m/z as the product ions. The ionization of ESI were following these conditions: spray voltage 3 kV; evaporation temperature 250 °C; capillary temperature, 300 °C; nitrogen as a 40 psi sheath gas pressure and Aux gas pressure 10 psi with argon gas. All operating conditions were set using the x-calibur 2.0 program.

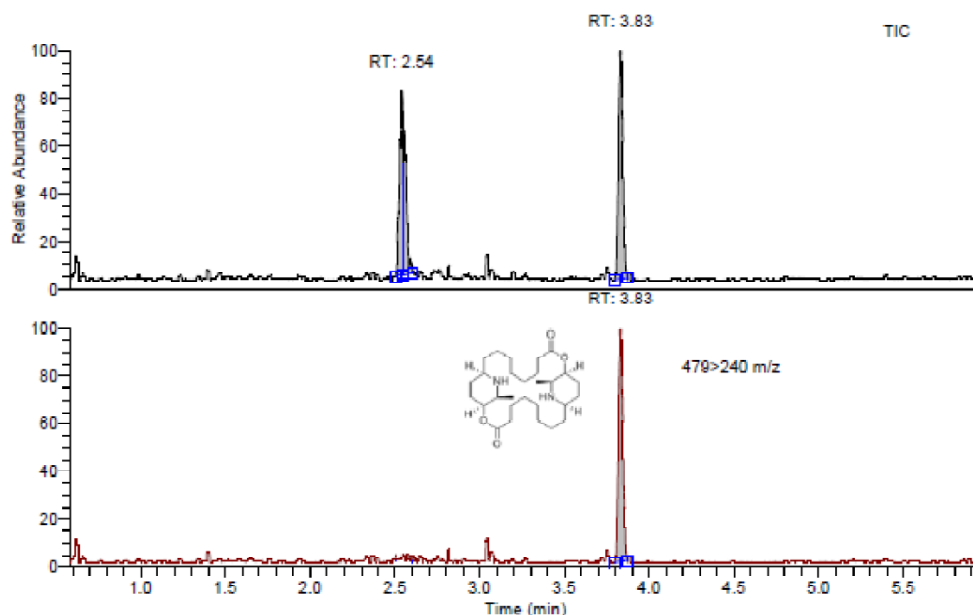


Fig. 1: Carpapine chromatogram of papaya leaves extract (*Carica papaya* Linn)

From the analysis of papaya leaves extract, there are carpapine compounds based on LC-MS/MS identification (Fig. 1). There are two chromatograms shown for carpapine identification. There are two peaks on the upper chromatogram as TIC (Total Ion Chromatogram), indicating that there are two compounds that have molecular ions of 479 m/z. In bottom chromatograms as XIC (Extracted Ion Chromatogram), the identification of the carpapine compound shows that there is one peak where the precursor ion is at 479 m/z and the product ion at 240. There is a peak in RT 3.83 minutes suspected as carpapine. The results of this study indicated the identification of alkaloid compound detected in papaya leaves extract.

Knowing the potential of alkaloid compounds contained in papaya leaves, they can be used as further study material for the utilization of chemical compounds as drugs (3). In general, alkaloids are often used in the medical treatment (5). Carpapine contained in papaya leaves has antitumors, anticancer and antimicrobes properties (2,5). Duke (6) reported that the secondary metabolite content of the papaya leaves is, among others, carpapine alkaloids and pseudocarpaineam which are piperidine group of alkaloids. The piperidine-type

alkaloid compounds that have anticancer activity and have anticancer mechanism by inducing apoptosis are flavopiridol compounds which are the resultant compounds of piperidine alkaloids with flavonoid compounds (7).

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