INTRODUCTION
Cancer is a group of cells that build mass of a tissue. It is delivered from developed tumor and transformed into a cancer cells that has uncontrolled proliferation (the outlaw cell). Cancer cells have a higher level of differentiation among the normal cells. These cells have angiogenesis ability and they invade the nearest cells or tissues. The condition could be severe when the cells begin to develop into other parts of the body, known as metastasis [1, 2]. It is estimated that there are approximately 7.6 million or 13% of the world population of deaths caused by cancer. This number is very high and according to the World Health Organization (WHO), in 2013, if there is no control, it can be estimated that in 2030 there are 26 million people will suffer from cancer and 17 million people will die from cancer. Cervical cancer is a type of cancer that leading cause of cancer deaths to four in the world after breast, lung, colon and rectum with a highest number of cases in Central America and South America, and Africa [3]. This cancer occurs in the cervix area and caused by infection with the Human Papilloma Virus (HPV). The prevention of cancer nowadays can be conducted by avoiding causative factors, such as smoking, exposure to sunlight and UV rays, exposure to radiation and other carcinogens. In addition, cancer can also be avoided by adjusting their lifestyle and diet [2, 4]. In addition to prevention, treatment has been done to prevent the cancer from spreading further and the treatment includes surgery, radiation, and chemotherapy. Unfortunately, these types of therapies give considerable side effects and cause excessive damage to normal cells [5]. It has been a long history that plants have been used for the treatment of cancer and are safer and less side effects than available chemotherapeutic agents. Mangosteen or Garcinia mangostana L is one of plants which has attracted much interest for evaluation of its pharmacological properties including cytotoxic activity against cancer cell lines (9-17). The ethanol extract of mangosteen inhibits proliferation of T47D and HeLa cell lines via NF-KB pathway inhibition [9]. Some compounds, α-mangostin, cowanin, rubraxanthon, cowaxanthon, cowanol and β-mangostin, in G. oliveri pericarps have cytotoxic activity against MCF-7 and DLD-1 cells, and α-mangostin shows the most potent activity [6]. In addition, the compounds contained in mangosteen peel extract has been proven to have anticancer activity on lung and stomach [7], antileukemia [8-11], breast cancer [12], and colon cancer [9]. In this study, cytotoxicity of the ethanol extract, n-hexane, ethyl acetate, and water fractions, and compounds isolated from mangosteen pericarp was examined against the HeLa cervical cancer cell lines by the Water Soluble Terazonium (WST) assay.

MATERIALS AND METHODS
Plant materials: Plant materials used in this study were pericarps of mangosteen or Garcinia mangostana L that has been taken from Desa Puspahiang, Tasikmalaya, West Jawa on August 2016. Specimens were identified in the Herbarium Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran and stored in the Herbarium Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran.

Chemicals: HeLa cells, RPMI medium 1640, fetal bovine serum (FBS), antibiotic (100 U/mL penicillin and 100 µg/mL streptomycin). Acetic acid, butanol, ethanol 96%, eter, alcohol, amonia 10%, gelatin 1%, sulfic acid, vanilin 10% in sulfuric acid, chloroform, magnesium, Chloride acid, FeCl3 1%, Mayer and Dragendorff reagent.

Other materials: 96-well plate (Thermo Scientific), TLC chamber, CO2 incubator (Thermo Scientific), conical tube (Thermo Scientific), separation funnel (PYREX), ELISA reader (Thermo Labsystems), evaporator, UV light λ366 and λ–254, Laminar Air Flow (Thermo Scientific), muserator, micropipette 1 mL and 200 µL (Gilson), microscope (Olympus CK40), neraca analitik (Mettler Toledo AL 204), oven (Memmert), petri dish flask (Thermo Scientific), capiler pipe, Pasteur pipette, sentrifugator (Thermo Scientific), spatel, liquid nitrogen tank (LOCATOR 4), waterbath, and laboratory glassware.

Methods
Determination of parameters of the extract
The parameters included organoleptic, calculation of rendement extract, water content, and the pattern of chromatography. These procedures were based on Indonesian Herbal Pharmacopoeae First edition on 2008.
**Phytochemical screening**

The extract obtained was subjected to a phytochemical screening procedure to identify secondary metabolites contained in the extract by a means of Farnsworth method (1966). The metabolites screened were alkaloid, flavonoid, tannin, saponin, quinone, monoterpen, sesquiiperpen, triterpenoid, and steroid.

**Isolation with Column Chromatography and Preparative TLC**

Isolation work of active compounds was done on the fraction having high cytotoxicity against HeLa cell lines using column chromatography. The fraction was subjected to preparative thin layer chromatography (TLC) using the mixture of n-hexane : ethyl acetate : methyl chloride (6 : 2,5 : 1,5) as development solvents. Two compounds, I-A and II-5B, were isolated. The compounds or isolates were identified by an analysis of its spectroscopic data (UV, IR, and MS) and compare of its data with those reported in the literatures.

**Identification of Isolates**

Isolates were analyzed using Ultraviolet-Visible spectrophotometry, infrared spectrophotometry, and Mass Spectroscopy and then the results were compared to literatures. Isolates were dissolved in methanol and placed in the cuvette. First, a blank sample was tested with the solvent. Analysis was performed at a wavelength of 300-700 nm. FTIR spectra were recorded using an IR-Prestige-21 (Shimadzu) spectrometer. Melting points were obtained on an electrothermal spectrophotometry, infrared spectrophotometry, and Mass spectrometer. Melting points were obtained on an electrothermal melting point apparatus (STUART-SMP10). UV spectra were determined on UV-Vis spectrophotometer (analytical Jena, specord-200).

Mass spectra were measured on Agilent 1100 Series LC-MSD-Trap-VL spectrometer using electron spray ionisation as an ion source type. Approximately 1 mg of sample was dissolved in methanol or chloroform, filtered to remove undissolved compound before it was injected into the mass spectrometer inlet.

**Cytotoxicity Test**

This test was conducted to determine the cytotoxicity of extract, fractions and isolates of mangosteen pericarp. This test was done by WST assay method comprising the following steps as mention below.

**Cells Preparation**

The inactive cells in the ampoule container taken from the liquid nitrogen tank and thawed quickly at 37 °C and then sprayed with 70% ethanol. Ampoule was opened and the cells were transferred into a sterile conical tube containing medium RPMI 1640. The cell suspension was centrifuged for 5 minutes, then the supernatant was discarded, added 1 mL medium containing 20% FBS, then grown the cell in tissue small culture flask, incubated in an incubator at 37 °C 5% CO2 for 24 hours. After 24 hours, the medium was replaced and grown the cell until confluent and enough for research. It is confluent.

After a sufficient number of cells, the medium was removed and cell’s colony were washed. The liquid part was disposed, then added 1ml or 2.5% trypsin. PBS 3 mL was added to balanced cell’s distribution, wait about 3-5 minutes to allow the trypsin to work well. Cells then transferred into sterile conical tubes and added RPMI 1640 to a volume of 10 mL and centrifuged at 3000 rpm for 10 minutes. Cells were washed twice with the same medium and counted the number of cells using a haemocytometer.

**Cytotoxicity test against HeLa cells (WST assay)**

The cell suspension in RPMI 1640 medium PRF of 50 mL (HeLa cell concentration of 1.0 x 105 cells / well) put in a 96-well plate and the plate was incubated for 24 hours in a 5% CO2 incubator. Then the sample was added into each well a different variation of the final content of the sample. Further plate was incubated for 24 hours in a 5% CO2 incubator at 37 °C. The end of the incubation medium in each well were removed and washed with PBS and then added 10 mL WST-8 cell counting solution. Plate was incubated again for 3 hours at 37 °C. Absorption was read using a microtiter plate reader at 550-600 nm [13].

**Data analysis**

Data was analyzed using IC50 determination. IC50 is the concentration required sample weeks to inhibit 50% growth of cancer cells or control cells.

### RESULTS

**Extract Parameters**

The extract of mangosteen pericarp was evaluated for organoleptic parameters, water content, and TLC profiles.

1. **Organoleptic parameters**

The organoleptic parameters evaluated were shown in Table 1.

**Table 1. The organoleptic test of ethanol mangosteen pericarp extract**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape</td>
<td>Condensed</td>
</tr>
<tr>
<td>Color</td>
<td>Brownish</td>
</tr>
<tr>
<td>Odor</td>
<td>Extract odor</td>
</tr>
<tr>
<td>Flavor</td>
<td>Bitter</td>
</tr>
</tbody>
</table>

2. **Water Content**

Water content check was performed towards the extract that reached constant weight. It was assumed that extract with constant weight has no longer content excess solvent, so that the extract would gave better water content check result. The amount of the extract that used for this water content check was 2 g. The extract was wrapped in aluminum foil with the top part was open to facilitate the entry of solvent, then it put in a round-base glass container 200 mL of re-distilled toluene. Distillation performed for 3 hours. After 3 hours of distillation, the water that contained in extract would be separated from the toluene. The obtained water volume was 0.15 mL so that the result of water content percentage calculation was 7.5%.

3. **Thin Layer Chromatography Evaluation**

The obtained extract was used to perform TLC check using GF 254 silica as stationary phase and chloroform: methanol (95:5) as mobile phase. This ratio was selected based on polarity of extract and its compound. It was following the principle of adsorption and partition that would give spot on the TLC result as shown in Table 2.

**Table 2. TLC results of mangosteen pericarp extract**

<table>
<thead>
<tr>
<th>Spot</th>
<th>RF</th>
<th>Visual</th>
<th>UV λ254</th>
<th>UV λ366</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.875</td>
<td>-</td>
<td>Purple</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>0.513</td>
<td>-</td>
<td>Purple</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>0.375</td>
<td>-</td>
<td>Blue</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>0.281</td>
<td>Yellow</td>
<td>Purple</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>0.175</td>
<td>Yellow</td>
<td>-</td>
<td>Blue</td>
</tr>
<tr>
<td>6</td>
<td>0.138</td>
<td>Yellow</td>
<td>-</td>
<td>Blue</td>
</tr>
</tbody>
</table>
**Phytochemical**

The phytochemical content identification was performed to identifying secondary metabolites types of the compound of mangosteen pericarp. The result of phytochemical was shown in Table 3.

### Table 3. Phytochemical Test of mangosteen pericarp extracts

<table>
<thead>
<tr>
<th>Secondary Metabolites Type</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Monoterprenoids dan Sesquiterpenoid</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Quinones</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
</tbody>
</table>

Information: (+) = detected (-) = undetected

The result of the phytochemical content identification showed that mangosteen pericarp herbal crude drug containing secondary metabolites such as polyphenols, flavonoids, and triterpenoids, quinone, and saponin.

**Fractionation by Liquid-Liquid Extraction and Column Chromatography Results**

The ethanol extract of mangosteen pericarps (100 g) was fractionated with a mixture of n-hexane-water and ethyl acetate-water, successively, to result in the n-hexane fraction (16.78), ethyl acetate fraction (44.45 g), and water fraction (14.61 g). The ethyl acetate fraction (10 g) was fractionated by gravity column chromatography method with silica gel 60 (0.040-0.063 mm) as stationary phase and n-hexane:ethyl acetate (7:3) as mobile phase. The TLC check was performed by comparing the collected fraction spot with α-mangostin standard compound spot. In the same mobile phase, the Rf of the α-mangostin standard compound was 0.275 and the Rf of the gartanin standard compound was 0.65. The results of the TLC check showed that there was some fraction that had the same Rf. The TLC check was performed by comparing the collected fraction spot with α-mangostin standard compound spot. In the same mobile phase, the Rf of the α-mangostin standard compound was 0.275 and the Rf of the gartanin standard compound was 0.65. The results of the TLC check showed that there was some fraction that had the same Rf. Meanwhile the spectrum the isolate II-5B showed that its absorptions were at 2848 cm⁻¹ (moderate intensity), 2966 cm⁻¹ and 2917 cm⁻¹ (moderate intensity), 3416 cm⁻¹ (moderate intensity), 3134 cm⁻¹ (moderate intensity), 1618 cm⁻¹ (High intensity), C=O on 1642 cm⁻¹ (moderate intensity), aliphatic C-H on 2966 cm⁻¹ and 2917 cm⁻¹ (moderate intensity), 2902 cm⁻¹ and 2846 cm⁻¹ (moderate intensity), and 2718 cm⁻¹ (moderate intensity). Other than that, the infrared spectrum of isolate II-5B showed that its absorptions were at 2848 cm⁻¹ (moderate intensity), 2966 cm⁻¹ and 2917 cm⁻¹ (moderate intensity), C=O on 1642 cm⁻¹ (moderate intensity), and C-O on 1283 cm⁻¹ (high intensity).

### Isolation by Column Chromatography and Preparative TLC Method Results

The condensed fraction (I) was used to perform another gravity column chromatography with smaller diameter of column, it was performed to purified the fraction. The column chromatography principal was the difference of the compound polarity. The condensed fraction (I) was fractionated by gravity column chromatography method with silica gel 60 (0.040-0.063 mm) as stationary phase and n-hexane : ethyl acetate as mobile phase. From the previous column chromatography which produces condensed fraction (I) had known that the compound was effectively eluted at solvent ratio 80:20. The condensed fraction (3.29 g) (I) was fractionated with n-hexane:ethyl acetate (80:20) as mobile phase. The amount of the mobile phase that had been used was 1.5 L. The fraction was collected each 50 ml into vial and used to perform TLC check with silica gel 60 F254 as stationary phase and n-hexane : ethyl acetate (7:3) as mobile phase. The TLC check was performed by comparing the collected fraction spot with α-mangostin standard compound spot. The results showed that fraction number 54-80 containing the target compound that had the same Rf. The TLC check was performed by comparing the collected fraction spot with α-mangostin standard compound spot. The results showed that fraction number 54-80 was combined into the same vial and labeled as isolate I-A. Isolation process was continued with preparative TLC with silica gel 60 F254 as stationary phase and chloroform : methanol (95:5) as mobile phase and it was detected under the UV 254 nm light. The same process was performed to condensed fraction (II) and the isolates labeled as isolate II-B.

### Purity

Isolate purity test was performed towards isolate I-A and isolate II-B by 2 Dimension TLC. The first mobile phase that used in the 2D TLC was n-hexane : ethyl acetate (7:3) as mobile phase and chloroform:methanol (95:5) as stationary phase. From the previous column chromatography which produces condensed fraction (I) had known that the compound was effectively eluted at solvent ratio 80:20. The condensed fraction (3.29 g) (I) was fractionated with n-hexane:ethyl acetate (80:20) as mobile phase. The amount of the mobile phase that had been used was 1.5 L. The fraction was collected each 50 ml into vial and used to perform TLC check with silica gel 60 F254 as stationary phase and n-hexane : ethyl acetate (7:3) as mobile phase. The TLC check was performed by comparing the collected fraction spot with α-mangostin standard compound spot. The results showed that fraction number 54-80 containing the target compound that had the same Rf. The TLC check was performed by comparing the collected fraction spot with α-mangostin standard compound spot. The results showed that fraction number 54-80 was combined into the same vial and labeled as isolate I-A. Isolation process was continued with preparative TLC with silica gel 60 F254 as stationary phase and chloroform : methanol (95:5) as mobile phase and it was detected under the UV 254 nm light.

### Spectroscopy Identification of isolated compounds

Isolate identification was performed using ultraviolet-visible spectrophotometry, it have been conducted to see the transition type experienced by these isolate electrons (Krull and Thompson, 2000). The ultraviolet spectrum of the isolate I-A showed that its maximum absorbance was λmax 263 nm, 270 nm and 323 nm. Meanwhile the spectrum the isolate II-B showed that its maximum absorbance was λmax 222 nm, 244 nm, 261 nm, 285 nm and 353 nm. As the spectrum showed that there was an absorbance in the ultraviolet area, it means that there was conjugated double bond in the structure of the isolate. Moreover, with the presence of 2 spectrums this referred to flavonoid compound. Absorbance range of flavonoid content compound was 350-385 nm on the first ribbon and 250-280 nm on the second ribbon [14].

The subsequent identification was performed using infrared spectroscopy. The infrared spectrum of isolate I-A showed that there was a strain of O-H on 3134 cm⁻¹ (moderate intensity), aliphatic C-H on 2966 cm⁻¹ and 2917 cm⁻¹ (moderate intensity), C=O on 1618 cm⁻¹ (High intensity), and C-O on 1287 cm⁻¹ (high intensity). Other than that, the infrared spectrum of isolate II-B showed that there was a strain of O-H on 3416 cm⁻¹ (moderate intensity), aliphatic C-H on 2966 cm⁻¹ and 2917 cm⁻¹ (moderate intensity), C=O on 1618 cm⁻¹ (High intensity), and C-O on 1287 cm⁻¹ (high intensity).
The results of cytotoxicity test expressed in IC$\textsubscript{50}$ values were calculated. With this little amount of reagent, it was titrated diluted from $100\mu$g/mL to detect the number of alive cell. The amount of cell in each well was measured by ELISA reader on wavelength 450 nm and 620 nm to detect the number of the alive cell. If the extract that obtained from the same sources was also tested, the compound I-A showed the highest cytotoxicity (IC$\textsubscript{50}$ : $10.58\mu$g/mL [22]). However, this IC$\textsubscript{50}$ was more active than the other plant extracts that reported by some researcher [23]. There might be other active compounds in the mangosteen pericarp having cytotoxicity against HeLa cell lines since other fractions showed the cytotoxic activity. Generally, semi polar fractions of some plants has good activity than non polar fractions as reported by previous study [24, 25].

The previous research showed that the xhantone derivates contained in the mangosteen pericarp extract had an anticancer activity against lung and gastric cancer[7], leukemia[8, 10, 11], breast cancer[12], and colon cancer [9].

**Conclusion**

The results of the research indicated that the ethanol extract, n-hexane fraction, ethyl acetate fraction, isolate I-A, and isolate II-5B had cytotoxic activity against HeLa cervical cancer cells, and the compound I-A showed the highest cytotoxicity.

**Acknowledgment**

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**Conflict of Interest**

Conflict of interest declared none.

**References**