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Aristolone content analysis in *ficus auriculata* as predicted apoptotic activity in hela cells

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ABSTRACT

Antioxidants and herbs have been scientifically proven to be one of the substances that can prevent cervical cancer. The incidence of cervical cancer in Indonesia is very high and is a vital cause of death of women in Indonesia. Every day 40-45 new cases emerge, which means 20-25 people die from cervical cancer in Indonesia. Several previous studies have shown that there are plants, fruits and seeds that contain high antioxidants and are believed to prevent and reduce pain due to cervical cancer. Besides, many herbs that also found in Indonesia are known to affect cancer cells. *Ficus auriculata* can find in Indonesia. However, not many people use the available natural resources that are suspected to prevent cervical cancer. This study was a preliminary study analyzed of *Aristolone* in Ara Fruit used as a prediction of apoptosis in HeLa cells. The results showed that the positive effect of *Aristolone* content in *F. auriculata* affected proliferation supported by previous studies.

1. Introduction

The incidence of cervical cancer in Indonesia is 16 / 100,000 women.(Ministry of Health, 2015). The number of data on cervical cancer in 2016 was 17.8 million people, in 2017 to 21.7 million people. It was an increase of 3.9 per cent of the number of people with cancer. It makes cervical cancer referred to as the number 1 female cause dead in Indonesia. Besides, this also makes number two in a large number of cervical cancer in sufferers world. It is quite alarming because most new cases rejected after an advanced stage and health efforts (Yin S, 2013). What often done is that successful treatment and therapy is still minimal.

Alternative medicine emerged as an attractive way to treat or cure cancer, and some plants and constituents have been developed because it is safe, effective, and cheaper to manage various types of cancer. Some medicinal plants found to contain active compositions capable of supporting cancer cell homeostasis. (Siegel, 2016). Indonesia has natural resources which contain herbal plants which are very easy to obtain. Some herbs that believed to be anti-cancer include Brotowali (Tinospora Cordifolia) (Polu, 2107). The study conducted was the effect of methanol extract of sapodilla fruit on mice that showed apoptosis in tumour cells in mice, as well as Figs cytotoxic effect (Khodarahmi, 2011). These fruits are easy to obtain in Indonesia, but the apoptosis effect is unknown.

This study focuses on one kind of Figs (Ficus auriculata). Recently, the study stated Typification of Ficauriculata complex (Ficus subsect. Neomorphe) discussed and F. beipeiensis treated as a new Synonym (Zhang, 2019). The reason is F. auriculata, F. oligodon, F. hainanensis, and F. beipeiensis compose a monophyletic group. This four species of this complex are with small genetic distances, shared haplotypes, and overlapped geographic distribution, and should be treated

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as a single species (Zhang, 2018). It uses for food and has socioeconomic importance, the near-ripe peeled or unpeeled fruits and young leaves of F. auriculata were sold such as in the north and central Vietnam (Mon, 2020).

In line with this study in apoptosis effect, some studies have stated about used of F. auriculata for an anticancer candidate. F.auriculata leaves extract has a cytotoxicity effect (Limanan et al., 2108), also isoflavone content in the root (Qi, 2017). For part of other plants need to be concern focus on an anti-cancer component. This study was a preliminary study to find out one of the substances in F. auriculata fruit which is suspected to have an apoptotic effect on HeLa cells.

2. Method

The design of this study used the method of observation and analysis of testing materials/samples. The sample in this study was 70% ethanol extract of *F. auriculata*. The sample tested by the method of analyzing *Gas Chromatography-Mass Spectrometry* (GCMS) by testing the presence of certain compounds according to the library of test equipment.

The steps used in this study:

1. Dried the Ficus auriculata
   It was done by a simple method, used a simple oven.

Fig. 1. The driying process

2. Processed the flour
   The flouring process also carried out by a simple method, namely by smoothing the dried fruit and sifting 80 mess.
3. Extracted
   Extraction carried out with 70% ethanol.

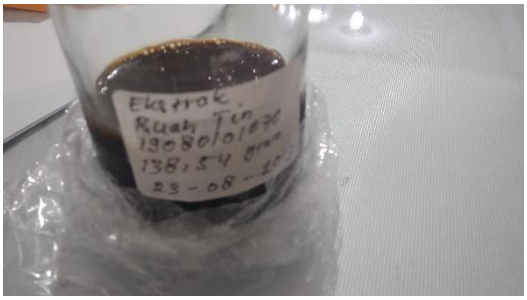

Fig. 2. *F. Auriculata* extract in 70% ethanol

4. GCMS Test
   GCMS testing conducted at the DKI Jakarta Provincial Health Laboratory (LABKESDA).

3. Results and Discussion

The results showed that there were Aristolone compounds in *Ficus Auriculata* with the following analysis:

Table 1. Result of GCMS test

Sample name	RT	Quality	Substance	Amount of Content
Ficus Carica	40.173	94	Aristolone	10.19%

Chemical formula : $C_{15}H_{22}O$

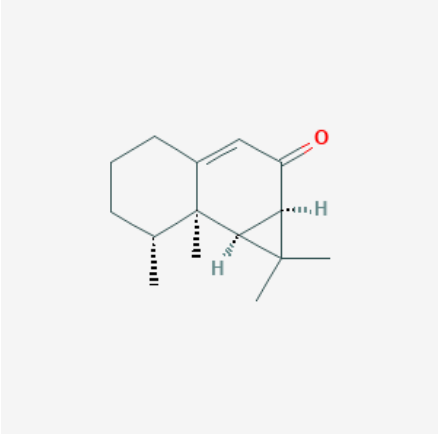

Fig. 3. 2 D Structure of Aristolone *(Pubchem,2020)*

Analysis of the results showed that there was about 10,19% Aristolone content in F. auriculata extract. Aristolone activities include potential inhibitors of the C1 complement component. Aristolone is the main constituent that produces cytotoxicity effects on cells. This in vitro study showed positive results (Marrelli, 2015). This is similar to the constituents contained in Cyperus longus. Experiments carried out in vitro Cyperus longus extract, fractions and essential oils (EO) on the MCF7 and PC3 cell lines. The chemical constituents of EO were identified using gas chromatography (GC) mass spectrometry (MS) analysis (Jamil & Ghani, 2017),(Siegel, 2016) The cells were cultured in RPMI-1640 medium and incubated with various concentrations of plant extracts and fractions. Cell viability was calculated by MTT test after 24, 48, and 72 hours exposure to (12.5-200 µg / ml) methanol extract, dichloromethane (CH2Cl2), ethyl acetate (EtOAc) and water fractions, and EO from plants. The percentage of apoptotic cells was determined using propidium iodide staining from DNA fragments with flow cytometry (peak sub-G1). Cytotoxicity checks were performed with IC50 values after 48 hours, respectively, 22.25 ± 4.25 and 12.55 ± 3.65 on PC3 and MCF7 cell lines. DNA fragmentation tests also confirm this data. This needs further research as a promising and preventive agent in the treatment of cancer (Memariani, 2016).

The same thing is also supported by Marreli et al's 2015 study, in which Aristolone is also contained in *Rosmarinus officinalis*. In the in vitro trial Aristolone contained therein has an anti-proliferative effect on the LoVo cell line with IC50 16.60 mg/ml. Aristolone stated as cytotoxic/anticancer potential (Ahmad, 2016), (Nogueira, 2020). Aristolone in this trial was found as much as 11.3%. This shows positive evidence that Aristolone in *Ficus auriculata* is also high, which is 10.19% higher than the Memariani et al 2016 trial where Aristolone contained 6.39%. The presence of cytotoxicity effects on Aristolone will tend that this active substance can cause apoptotic effects as well, especially on HeLa cells. (Gama – Sitosterol, 2019). This strengthened with the statement that F. Auriculata displayed more potent anti-proliferative activity than chemotherapy medicine (Ghani et al., 2019), (Putthawan,Poaeman, 2017), (Shao, 2014). Besides it also contain an

antioxidant component(phenolic) and DPPH Free Radical Scavenging Activity (Wong, 2020) and antibacterial (Shao, 2020).

4. Conclusion

The initial conclusion in this study was, the Aristolone content in F. auriculata extracts is predicted to have an apoptotic effect on HeLa cells because the initial activity of Aristolone was known to have a cytotoxic effect on cells.

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