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Cytotoxicity and Histopathological Analysis of *Capsicum frutescens* via *Artemia salina*

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ABSTRACT

Capsicum genus contains many species of sweet and hot chili peppers. *Capsicum frutescens* is one of the important chili peppers that used as flavor, aroma and spices in the national cuisine. It has also been used as medicinal agents. The phytochemical compound which is responsible for the pungency is capsaicin. For control safety consumption, therefore the toxicity or side effect needs to be investigated. The aim of this research evaluated the amount of total phenols contents from different factors such as colors (red and green) and fruit parts (pericarp, capsaicin gland or placenta, and seed) of *C. frutescens* aqueous extraction in 1, 3, 5, 10 and 24 hours. The highest total phenolic content was shown in the 24 h extraction. So, this extraction time was used to investigate the cytotoxicity and histopathological alteration by using brine shrimp, *Artemia salina* as an animal model. Ten adults *A. salina* were incubated at room temperature for 24 h with various concentrations of chili. The mortality number of *A. salina* was recorded and the median lethal concentration value was calculated. The highest toxicity was reported in the green pericarp group. The primarily target organ was the intestine of brine shrimp. Enterocytes showed abnormal morphology such as edema, hyperplasia, disorganized arrangement, and finally necrosis. Moreover, the pericarp of the green chili showed the most severe results. It can be concluded that different colors and fruit parts of *C. frutescens* shows different amounts of phenolic content and correlates with cytotoxicity leading to the severity of the histopathological alterations in *A. salina*.

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Introduction

Capsicum frutescens, also known as bird chili, is a member of flowering plants in genus Capsicum, Solanaceae family. This genus is native to Central and South America and comprises more than 27 species of sweet and hot chili peppers (Ibiza *et al*., 2012). *C. frutescens* is one of domesticated consumption species, which includes *C. annuum, C. chinense, C. baccatum*, and *C. pubescens* (Singh, 2007). In the global market of spice, the crop of *C. frutescens* is an important agricultural production. It is widely used as a condiment or coloring agent in many foodstuffs. In 2016, the Food and Agriculture Organization reported that the total global production of peppers including both dry and fresh green chilies were more than 33.2 million tons, and the main producing region is Asia.

Pungency or hot taste is a characteristic of *C. frutescens* fruit due to activity of capsaicinoid, a group of substances that specifically present in the members of the genus Capsicum (Asnin and Park, 2015; Lu *et al*., 2017). Moreover, it has been reported that capsaicinoid is showed higher level in *C. frutescens* than in *C. annuum* (Wahyuni *et al*., 2011) and higher in placental tissues than in pericarp and seeds of the dried fruits (Cisneros-Pineda *et al*., 2007).

Concerning the important bioactive compounds and chemical compositions, fruits of *C. frutescens* are rich source of carotenoids, ascorbic acid, tocopherols, flavonoids and capsaicinoids (Howard and Wildman, 2007). A study showed that many of these compounds have an antioxidants effect (Padayatty *et al*., 2003), associated with possess of anti-cancer, anti-inflammatory (Chapa-Oliver and Mejia-Teniente, 2016), and antimicrobial properties (Füchtbauer *et al*., 2021), In addition, the chemical extracted from *C. frutescens* has been applied in pharmaceutical industries, as it played a role in pain relief (Chung and Campbell, 2016)and weight reduction (Okamatsu-Ogura *et al*., 2015). It also had cardiovascular and gastrointestinal protective effects (Sun *et al*., 2016). For control safety consumption, therefore the toxicity or side effect needs to be investigated.

The present study aimed to evaluate the amount of total phenols contents from different factors such as colors (red and green) and fruit parts (pericarp, capsaicin gland or placenta, and seed) of *C. frutescens* aqueous extracted and to investigate the cytotoxicity effect and histopathological alteration of its extract to *A. salina*.

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Materials and methods

Capsicum extracts

The experiment groups were named by two letters: the first letter was colors (R-red and G-green), and the second letter was plant parts (G-capsaicin glands, P-pericarp and S-seed). The extraction procedure was determined by modifications method of Adjé *et al*. (2012). One gram of each part of plant was extracted with 100 ml of distilled water at 180 rpm for 1, 3, 5, 10 and 24 h at room temperature. The whole mixture was centrifuged at 4,000 rpm for 10 min and filtered through a fresh gauge plug. Finally, the clear supernatant was kept in -20°C and used as a stock solution for future experiments.

Total phenolic compound measurement

The total phenolic content was determined according to Folin-Ciocalteu method with minor modification of Ainsworth and Gillespie (2007). The 50 µl at concentration 2.5 mg/ml of each extract was mixed with 250 µl of 10% Folin-Ciocalteu phenol reagent in distilled water and 200 µl of 0.7 M sodium carbonate. The 4.5 ml of distilled water was added and incubated at room temperature for 2 h in dark condition. After that, the mixture was measured total content of phenolic compound in triplicate at 765 nm by using spectrophotometer. The standard solution in this study was gallic acid in ethanol. It was prepared at 0.625, 1.25, 2.5, 5 and 10 mg/ml for plotting the standard curve. The extract was calculated as mg of gallic acid equivalent (GAE) based on the linear equation of calibration curve analyzed by Microsoft Excel Software.

Brine shrimp lethality bioassay

The time extraction that shown the highest amount of total phenolic was used for the brine shrimp lethality bioassay. This assay is determined the cytotoxicity effect base on the killing ability of *C. frutescens* crude extract on brine shrimp (*Artemia salina*). This method followed by Meyer *et al*. (1982). The plant extraction was diluted to 0, 10, 100 and 1000 mg/L by mixing with 3.5% NaCl. Ten of *A. salina* were in 20 ml of each concentration of the extract and maintained at room temperature throughout the test. The experiment was done in triplicates. The mortality was recorded for 24 h of exposure. Animal was considered dead or moribund if it stopped moving for a prolonged period even after gentle probing with a small spatula. The LC50 was analyzed by the Probit method of Finney (1971) using the SPSS 22.0 (Statistical Package of Social Sciences) software. It estimated the lethal concentration and the slope of the regression line with its confidence interval ($p<0.05$).

Histopathological analysis

For histopathological study, brine shrimp was exposed with each plant extract in 50% of 24 h-LC50 concentration. The procedures for light microscopy were performed following the methods of Humason's animal tissue techniques (Meyer *et al*., 1982). Briefly, the tissues were fixed in Davidson's Fixative for 24 h. Then, they were dehydrated through a series of increase percentage of ethanol to remove the water in the tissues and cleared with xylene, infiltrated with embedding agent, paraffin, which was like a candle wax to mold in the tissue. Next, they were aligned carefully in blocks using melted paraffin at the embedding station. The paraffin blocks were sectioned at 5 ≒m thickness using a rotary microtome and placed on a glass slide. For staining, the slides were deparaffinized before and running through the reverse process from xylene to alcohols to water. Finally, they were stained with hematoxylin and eosin. The stained glass slides were examined for tissue abnormalities in the brine shrimp using the Olympus CX31 light microscope and photographed by a Canon EOS 1100D digital camera.

Results

Total phenolic content

The total phenolic content of each part of *C. frutescens* were determined after extraction at 1, 3, 5, 10, and 24 h (Fig. 1). The values of the phenolic concentration in RG and RP group were significantly increased depending on the extraction times. However, GG, GP, GS, and RS were not significantly different when compared between different extraction times, which total phenolic at 24 h were 32.03, 25.01, 16.35, and 13.36 mg/g GAE, respectively. Besides, the group of *C. frutescens* with red color and placenta region part (RG) showed the highest amount of phenolic compound, was 50.01 mg/g GAE and was significantly high at 24 h extraction ($p \leq 0.05$).

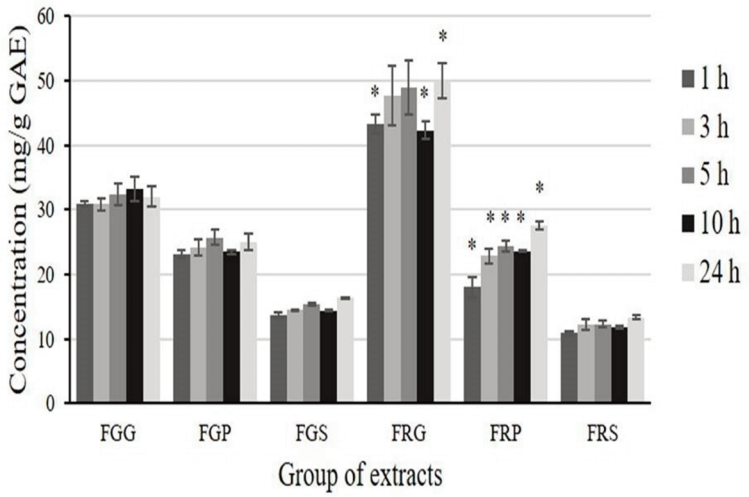

Fig. 1. The amount of total phenolic content in each extracted group with variable times. The values of phenolic concentration of RG and RP extracted were increased with time dependent. * is p-values between 24 h extracted and other extraction times in same group that significant at $p \leq 0.05$.

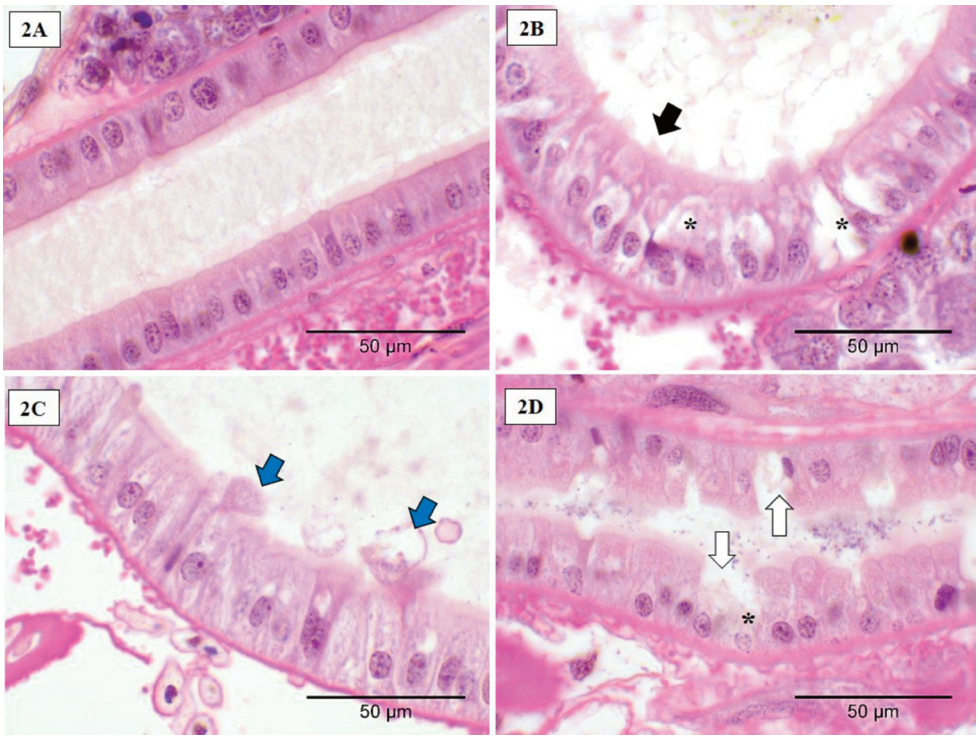

Fig. 2. Microscopic appearance of *Artemia salina* in control group (2A) showing single ciliated columnar epithelial lining. In GP group, (2B) showing epithelium edema (*) and brush border disruption (black arrow), (2C) showing bubbles cytoplasm (blue arrow), (2D) showing epithelium cells disorganized (white arrow) with necrosis presented by karyorrhexis nucleus (*).

For RP, its total phenolic was 27.55 mg/g GAE at 24 h. The correlation value (R2) was indicated at 0.9621 and the linear equation is Y = 0.1198ln(x) - 0.2589.

The 24-h LC_{50} of C. frutescens

After 24 h exposure, the motility rate was recorded, and it was found that the trend of cytotoxic effect was increased with dose dependent. The 24-h LC50 result for green color groups GG, GP, and GS were 1108.79, 605.88, 11988.81 mg/L, respectively. The 24-h LC50 result for red color groups, RG, RP, and RS were 2422.19, 1158.42, and 8683.19 mg/L, respectively. The toxicity level was following GP> GG> RP> RG> RS> GS.

Histopathology analysis of A. salina

The histopathological alterations were observed in the intestinal tract (enterocyte) of *A. salina* (Fig. 2). The control group showed normal ciliated simple columnar epithelial lining (Fig. 2A). The GP group had a major abnormality including edema, hyperplasia, disorganized arrangement, and necrosis. For edema, the epithelial cells showed swelling with pallid and enlarged cytoplasm, with disruption of the brush border (Fig. 2B). Next, the structure of the epithelial cell was lost with protruded elongations and fall out of bubbles cytoplasm into the lumen (Fig. 2C). Finally, disorganized of cells lining and necrosed cell were present (Fig. 2D). The alteration of epithelial cell response to cell injury and cell death were observed.

Discussion

C. frutescens is a one of important economic crops that widely used in many consumers and industrial product. Many of chemical substances in its fruit can use in pharmaceutical research and manufacturing process, especially the members of phenolic compound group.

In the present study, we observed and found the correlation between total phenolic compound in various factors of *C. frutescens*. The results were significantly the highest in red color and placenta region part. The previous report showed that amounts of capsaicin, a one of major phenolic content in Capsicum fruit, was found higher in placental tissues than pericarp and seeds of the dried fruits (Cisneros-Pineda *et al.*, 2007). The color represents the fruit age and showed an increase trend of capsaicinoid content from young green color to the older red color fruits (Mueller-Seitz *et al.*, 2008).

However, the chemical content of *C. frutescens* also has toxicity effect, especially capsaicin. Besides, it had been reported about acute oral toxicity and short-term inhalation toxicity. To observe its effect, we select the brine shrimp (*A. salina*) as an animal model due to the advantages of their sensitivity to aquatic pollution and toxicity represented by lethality and histopathological lesion. In general, the intestinal tract or enterocyte is a main absorption area of *A. salina* that will present the pathological lesion when they get pollutions or toxicity from the environment (Libralato *et al.*, 2016). In this study, the pericarp of *C. frutescens* with green color showed the strongest toxicity effect. It was confirmed by histopathology investigation. The enterocyte cells of *A. salina* have shown edema, hyperplasia, disorganized arrangement, and necrosis that confirmed the toxicity of *C. frutescens* extracts, which is responsible for processes of cell injury and cell death.

Conclusion

This study showed that the different factors of *C. frutescens* including color and plant parts are correlated with amount of total phenolic compound and influence cytotoxicity of *A. salina*. However, further research could be investigated to identify the active substances, and how they elucidate their effects.

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Conflict of interest

The authors declare that they have no conflict of interests.

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