IMMUNOMODULATORY ACTIVITY OF FRACTION EXTRACT FROM SWEET CORN SEEDS (Zea mays L.) ON MICE PERITONEAL

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Sweet corn (Zea mays L.) contains lutein that have potential as an immunomodulatory agent by improving immune system function. This study was aimed to determine the potential of sweet corn extract lutein as an immunomodulatory. Identification of lutein in the lutein fraction from sweet corn seed by TLC showed that the fraction contains lutein. This TLC data was confirmed using HPLC analysis. Identification of the functional group in the lutein extract using FTIR showed similar results with the lutein standard, i.e. there was a group C=C (Alkenyl), C-C (alkyl), -C=C (aromatic), and -OH (Hydroxyl). Immunomodulatory activity test was performed in vivo using mice peritoneal induced by Staphylococcus aureus. The lutein fraction with doses of 0.15, 0.3, 0.6, and 0.9 mg/kg BW/day. The results showed that the fraction were active as immunostimulant.

Keywords: Sweet corn (Zea mays L), lutein, immunomodulatory, phagocytosis, macrophage

1. INTRODUCTION

Immunity is a resistance to diseases, including infectious diseases. The activity of the immune system can be decreased due to various factors, such as age or illness. The presence of compounds that can increase the activity of the immune system is very useful to overcome the decline in immune system activity. One example of immunostimulant agent is lutein in sweet corn kernels [1]. Lutein is a natural pigment belonging to the carotenoid that is easily found naturally in fruits, vegetables, and also in the seeds of sweet corn (Zea mays L, 0.41 mg/100 g fresh weight) [5]. Carotenoids act as antioxidant that increase immunity. Its other benefits are to prevent eye-macular degeneration (cataracts), to protect skin from UV radiation, and to prevent degenerative diseases due to aging [2]. Previous studies reported that lutein has immune regulatory activity. Mice given lutein has increased in the proliferation of lymphocytes in response to phytohemaglutinin (PHA) and has increased in the production of antibodies in respond to T dependent antigen cells. Lutein and zeaxanthin compounds have physiological functions, i.e. protecting cells and tissues from oxidative damage and stimulating the immune system. Further research showed that 10 mg of lutein consumed per day by cats for 12 weeks led to an increase of the percentage of CD4+ and CD21+ lymphocytes, plasma concentrations of IgG, and NK cell activities. The results showed that lutein could stimulate cell-mediated immunity and humoral immunity in cats conducted in vivo [3,4]. This research was focused to examine lutein fraction from the seeds of sweet corn (Zea mays L.) as immunomodulator agent in macrophage cells obtained from mice peritoneal fluid. Based on a previous research on cats, the conversion of lutein dose was at 0.3 mg for the mice [4].

2. METHODS

This study used sweet corn (Zea mays L.) obtained from Bogor, West Java. Animals used in this study was DDY strain mice (aged 4 months, weight 22-24 g). Before treatment mice...
were adapted for one week. Bacteria \textit{Staphylococcus aureus} with a density of $10^8$ cells/mL were obtained from the Faculty of Veterinary of Bogor Agricultural Institute, West Java.

\textbf{Extraction Lutein From Sweet Corn Seeds (Zea Mays L.)}

One hundred gram of sweet corn seed powder was macerated with 1L of n-heksan for 48 hours. The extract was collected and evaporated to dryness by rotavapor. This extract was mixed with isopropanol and heated 60°C NaOH solution (50%) was added slowly at 60°C with stirring for 90 min. The saponified mixture was allowed to cool and then diluted with Deionized Water. The mixture was allowed to stand for approximately 60 min followed by addition of 4 times (v/v) deionized water. The precipitate was collected by centrifugation and dried under vacuum incubator at 40°C.

\textbf{Analysis by Thin Layer Chromatography.}

Sample of lutein fraction from Sweet corn seeds (\textit{Zea mays L.}) was analyzed using TLC on silica gel 60 GF$_2$54, eluted with n-hexane-chloroform-acetone (6:2:2).

\textbf{Analysis of Lutein from Sweet corn (Zea mays L.) by High Performance Liquid Chromatography (HPLC).}

Lutein fraction solution injected into HPLC instrument. Instrument condition was as follows: Column SunFire™ C18 5 µm (4.6 x 150 mm), Mobile phase Methanol; Acetonitrile (70:30), Detector PDA@450 nm and Flow rate 1.0 ml/min.

\textbf{Identification of Lutein by FTIR}

FT-IR analysis of lutein fraction from Sweet corn (\textit{Zea mays L.}) was done for functional groups similarity.

\textbf{Mice Oral Treatment.}

The experiments were carried out with 7 treatment groups, each group consisted of 4 mice. Each group was given treatment of 0.5 mL (oral) every day for 14 days. Group I was given Stimuno (positive control), group II was given non-cholesterol oil (negative control), group III was given distilled water (normal control), group IV-VII were given lutein fraction doses of 0.15, 0.3, 0.6, and 0.9mg/day, respectively.

\textbf{Phagocytosis Test.}

Mice were infected with bacteria \textit{S. aureus} suspension intraperitoneally, then incubated for one hour. Mice were then euthanized, dislocated, and their stomachs were dissected. Peritoneal fluid containing macrophages was pipetted stained with Giemsa and further observed under a microscope. Macrophage phagocytosis activity and capacity was calculated by one-way ANOVA, followed by Duncan test.

\textbf{3. RESULTS}

\textbf{Determination Of Water Content By Karl Fischer.}
The water content of sweet corn seed powder was 9.31%. This result qualifies for simplicia powder according to the Indonesia Herbal Pharmacopoeia (below 10%).

\textbf{Lutein Extraction of Sweet Corn Seed.}

Yield of sweet corn lutein extract obtained was at 1.035% with a value of DER-native was at 96.62.

\textbf{Analysis Of Lutein Using Thin Layer Chromatography.}

Results showed that there was yellow spotting on samples of sweet corn seed lutein which was the same with the standard reference of lutein with \textit{R}$_f$ value of 0.61 and \textit{hR}$_f$61.

\textbf{Lutein Analysis Using FTIR.}

Analysis of the comparative standard lutein compounds and lutein extract of sweet corn seeds with a \textit{fourier transform} infrared spectrophotometer (FTIR) showed a similar infrared spectra (Figure 1).
Figure 1. The infrared absorption spectrum of Lutein standard (left) and lutein extract of sweet corn seed (right).

Table 1. Analysis of spectrum of lutein standard and lutein extract of seed sweet corn (*Zea mays* L.) by FTIR Waves (cm⁻¹)

<table>
<thead>
<tr>
<th>Lutein BP</th>
<th>Lutein Extract</th>
<th>Reference</th>
<th>Function Cluster</th>
</tr>
</thead>
<tbody>
<tr>
<td>717,47</td>
<td>723,26</td>
<td>675 - 995</td>
<td>Alkenyl C = C</td>
</tr>
<tr>
<td>847,66</td>
<td>844,76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>930,59</td>
<td>923,84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>987,49</td>
<td>958,56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1325,97</td>
<td>1350,08</td>
<td>1340 - 1470</td>
<td>Alkyl C - C</td>
</tr>
<tr>
<td>1351,04</td>
<td>1349,87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1406,97</td>
<td>1405,80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1441,69</td>
<td>1469,66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1469,66</td>
<td></td>
<td>1600 - 1680</td>
<td>Aromatic - C = C</td>
</tr>
<tr>
<td>1662,52</td>
<td>1664,45</td>
<td>3300 - 3600</td>
<td>Hydroxy - OH</td>
</tr>
<tr>
<td>3370,37</td>
<td>3317,34</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3345,30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3422,45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Lutein Analysis Using High Performance Liquid Chromatography.**
Phytochemical analysis was done by comparing the HPLC profiles of the extract with lutein standard reference (Figures 2).

Figure 2. HPLC Chromatogram of lutein standard (left) and Sweet Corn Seed Lutein (right).

Table 2. Results of lutein standard and lutein fraction of *Zea mays* L by HPLC

<table>
<thead>
<tr>
<th>Name</th>
<th>Retention Time(min)</th>
<th>Area</th>
<th>Lutein Level (bpj)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Lutein Standard</td>
<td>3.681</td>
<td>112524</td>
<td>10</td>
</tr>
<tr>
<td>2 LuteinFraction</td>
<td>3.635</td>
<td>8816</td>
<td>0.78</td>
</tr>
</tbody>
</table>

**Phagocytic activity and capacity of macrophages.**
Phagocytic activity of macrophages of each group listed in Figure 3.
Figure 3. Percentage macrophage phagocytic activity of lutein fraction

An increase in phagocytic activity was observed after the administration of lutein fraction for 14 days with a dose of 0.15mg/mice, 0.3mg/mice, 0.6mg/mouse, and 0.9mg/mouse per day in a row was 16.51%, 30.66%, 42.45% and 52.36%. Figure 4 shows a fairly good linear relationship between the dose of the fraction with the phagocytic activity. The higher the dose of lutein, the greater the increase in phagocytic activity.

Figure 4. The increase in phagocytic activity in the treatment of various concentrations of lutein fraction from seeds sweet corn (Zea mays L.) for 14 days.

4. DISCUSSION
Sweet corn (Zea mays L.) was extracted and further separated to obtain lutein fraction. The FT-IR analysis was done and the functional groups association were determined. The FTIR spectra showed functional groups of –OH, -CH2, -C-O- and -C=O- (Table 1). Table 2 shows HPLC analysis of lutein standard reference produces chromatogram with a peak in the a retention time of 3.681. Analysis of lutein fraction from sweet corn seeds shown in Figure 2 and Table 2 indicated a peak area at the retention time of 3.635 minutes. The standard compound lutein in the chromatogram, there are two peaks in the standard samples of lutein and lutein fraction from sweet corn seeds. This because they were not pure. Activated macrophages cells were given lutein fraction doses of 0.15, 0.3, 0.6, and 0.9mg/day. Stimuno was used as a positive control. The activity of the group I was (84% ± 2.08); Group II (53% ± 0.82); group III (51% ± 1.41); group IV (61.5% ± 1.91); V group (69% ± 1.25) group VI (75.5% ± 2.08), and Group VII (80.75% ± 1.89). Duncan test shows that there was no difference between the distilled water control group and vegetable oil.
control group. Lutein fraction from the seeds of sweet corn showed a different activity. The higher the dose of lutein fraction, the higher the activity.

5. CONCLUSIONS
- TLC, HPLC and FTIR analysis indicated that the tested fraction from sweet corn contains lutein.
- The administration of lutein fraction with various doses showed distinct activity. The higher the dose of lutein extract, the higher the activity. This indicated that the lutein fraction from the Sweet Corn seeds can be used as an immunomodulator agent.

6. REFERENCES
7. Ellis, R, Giemsa's Staining Protocol for Tissue Sections. 2007. IMVS Division of Pathology Queen Elizabeth Hospital.