The Application of Experimental Based Guided Inquiry Model in Human Excretion System Material to Improve Students’ Science Process Skill

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Abstract. The aim of this research is to find out the improvement of students’ science process skill through the application of experimental based guided inquiry model in human excretion system. This research was conducted at SMA Negeri 1 Indrapuri Aceh Besar from April to June 2014. It used experimental based guided inquiry method with class of IPA\textsubscript{2} (n=25 students) as experiment class and IPA\textsubscript{3} (n=25 students) as control class. The application of experimental based guided inquiry model was analyzed by using science process skill rubric for the experiment class at the time of experiment and discussion with observation checklist for control class. The result shows that experimental based guided inquiry model improves science process skill of students of the experiment class. The skill of students of class of IPA\textsubscript{2} (n=25 students) has improved from enough to very good, while the skill of class of IPA\textsubscript{3} (n=25 students) has not improved from enough category. It can be concluded that students who were taught by using experimental based guided inquiry model has better science process skill than those were taught by discussion.

Keywords: Experimental Based Guided Inquiry Model, Science Process Skill, Excretion System.

INTRODUCTION

The scientific approach is the approach used in the curriculum of 2013. To enhance scientific approach, reasoning in finding is needed. To be called scientific, it has to rely on proofs of objects that are observable, empirical, and measurable with specific reasoning principles [2,4]. One of learning models that goes along with scientific approach is the inquiry model. Guided inquiry model is a model where students are guided to find a conclusion from a series of activity, as if the students acquired the knowledge by themselves [8]. Guided inquiry model is suitable to enhance students’ science process skill because it is related to skills they learn in research such as observing, formulating the research question, hypothesis, planning the experiment, conducting the experiment, collecting data, and drawing the conclusion [12]. That way the learning phases in experimental based guided inquiry can train the students’ science process skill. This model is well planned, truly instructionally controlled so the students can understand the material taught by teachers more comprehensively [6,1].

Science Process Skill (SPS) is a complex ability used by scientists in conducting scientific research in learning process [3,5]. Science process skill is a series of measurable activities from an experiment. Experiment is the best way to develop process skill [9,7] Experiment is an important activity in learning process. This activity is done in support of achieving the learning objectives. Some objectives cannot be achieved by the students and it can affect the outcome of their study [11,15]. Biology learning nowadays is still focused on teacher as the main character in teaching-learning process (teacher centered) not student oriented. It is still far from reaching the goal of curriculum 2013, which are to accelerate and motivate students to be smarter, more creative and active in learning.

MATERIALS AND METHODS

The research was conducted at SMA Negeri 1 Indrapuri located on Jalan Banda Aceh-Medan Km. 27 Indrapuri Aceh Besar. It was carried out in the even semester of academic year 2013/2014, from April 5\textsuperscript{th} to May 5\textsuperscript{th} 2014. Observation checklist is an instrument used to measure students’ science process skill during experiment in experiment class, and discussion observation check list in control class. Population in this research is 75 students of class XI IPA at SMA Negeri 1 Indrapuri consisting of 3 classes; XI IPA1 (n=25 students), XI IPA2 (n=25 students), and
XI IPA3 (n=25 students). Sample in this research are students of class XI IPA2 (n=25 students) as experiment class and XI IPA3 (n=25 students) as control class. Sample was chosen through purposive sampling. It is the act of choosing sample based on certain consideration [13]. In this research, the researcher used two classes: experiment class and control class. Experiment class is the class that used experimental based guided inquiry model the learning process, while control class used discussion in learning. The experiment design used in this research is based on guided inquiry. Observation form was established to assess science process skill during experiment, which were examining urine pH, glucose in urine, CO₂ and H₂O excretion from lungs, testing body temperature and gall in experiment class. Whereas in control class, the observation sheet was used in assessing science process skill during discussion which were about kidneys, lungs, skin and liver. Assessment scale ranges from 1 to 4. The numbers mean: 1 = poor, 2 = fair, 3 = good, 4 = very good.

\[
\text{Result} = \frac{\text{Total Student's Score}}{\text{Maximum Score}} \times 100
\]

**Table 1. Science Process Skill Observation's Result Criteria**

<table>
<thead>
<tr>
<th>Score</th>
<th>Result</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>28-36</td>
<td>81-100</td>
<td>A</td>
</tr>
<tr>
<td>19-27</td>
<td>61-80</td>
<td>B</td>
</tr>
<tr>
<td>10-18</td>
<td>41-60</td>
<td>C</td>
</tr>
<tr>
<td>0-9</td>
<td>20-40</td>
<td>D</td>
</tr>
</tbody>
</table>

**RESULT AND DISCUSSION**

**Science Process Skill Result of Experiment Class.** Data from analyzing science process skill of students of experiment class has improved during the implementation of guided inquiry while experiment. The improvement occurred in all science process skill observed. It was observed in every experiment. The overall data analyzed from every experiment is shown in (Figure 1).

**FIGURE 1.** The total scores versus the experiment.
The Average Improvement of Science Process Skill. Based on the analysis, every indicator of students’ science process skill in every experiment has improved. The improvement is shown in Figure 2.

Figure 2 exhibit that the average scores in each experiment has improved. The average score of examining urine pH experiment 60 which is fair, the average score of examining glucose in urine experiment 68 which is, the average score of examining CO₂ excretion experiment 76 which is good, the average score of examining H₂O excretion experiment 81 which is very good, the score of examining body temperature experiment 82 which is very good, and the score of examining gall pH experiment 84 which is very good. Thus, experiment is a method that can improve science process skill, since by doing experiment students can develop the basic skills of experiment. It
becomes a mean of achieving the goal of science learning, which are process and product oriented. Experiment is the best mean in developing science process skill. 

**Science Process Skill Result of Control Class.** Data from analyzing science process skill of students of control class shows no improvement during learning by discussion. Science process skill indicators observed during learning has not improved significantly. The data analyzed from every experiment is shown in (Figure 3).

![Graph showing science process skill results](image-url)

1= observing indicator  
2= classifying indicator  
3= predicting indicator  
4= questioning  
5= hypothesizing indicator  
6= planning group discussion indicator  
7= using theory from various sources indicator  
8= implementing concept indicator  
9= communicating indicator  

Discussion 1 = kidneys  
Discussion 2 = lungs  
Discussion 3 = skin  
Discussion 4 = liver
Figure 3 shows that science process skill measured during discussion has not improved significantly. However, the indicator which result is about to be significant are predicting and using theory from various sources indicators. The improvement of using theory from various sources indicator was affected by material worksheet used by students during discussion. Based on Figure 4 it is known that, the average score in every discussion has not improved significantly. The average score of discussion about kidneys is 58, which is fair, the average score of discussion about lungs is 68, which is good, the average score of discussion about skin is 70 which is good, and the average score of discussion about liver is 60, which is fair.

![Figure 4](image)

**FIGURE 4.** The Improvement of Average Score of Science Process Skill in Every Experiment

**CONCLUSION**

Students who were taught by experimental based guided inquiry model have had very good science process skills, whereas those who were taught by discussion have had fair science process skill.

**REFERENCES**


Cytotoxic Activity of Ethyl Acetate Extract of *Calotropis gigantea* L. Stem Bark and its Fractions against P388 Cells

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Abstract. The present study deals with the cytotoxic activity of ethyl acetate extract of *Calotropis gigantea* L. stem bark and its fractions such as A, B, C, D and E fractions on murine leukemia cell line P388. Qualitative screening of ethyl acetate extract of stem bark of *Calotropis gigantea* L. for secondary metabolites showed the presence of phenolics, alkaloids, steroids, flavonoids, saponins and coumarins. Cytotoxic activity of the ethyl acetate extract of this plant and A, B, C, D and E fractions was conducted on P388 cells through MTT assay, with IC⁵₀ value 57.05 μg/mL, 60.37 μg/mL, 55.65 μg/mL, and 58.26 μg/mL respectively, while D and E fractions less active. However the potential of the active fractions as indicated by the results in this study needs to be further investigated.

Keywords: *Calotropis gigantea*, secondary metabolites, cytotoxicity, P388 cells.

INTRODUCTION

Tumors and cancers are classified as diseases those are potentially dangerous to human life. The World Health Organization (WHO) reported deaths from the cancer by about 13% every year [1]. The major causes of cancer are smoking, dietary imbalances, hormones and chronic infections leading to chronic inflammation [2]. According to the International Agency for Research on Cancer (IARC) in 2008 worldwide, it was estimated that there were 12.7 million new cases, 7.6 million deaths; of these number, 56% of the cases and 64% of the deaths occurred in the economically developing countries [3,4]. The success of cancer therapy is still relatively low, this is due to limitations in the use of anticancer associated with safety, because almost all anticancer not only kill the cancer cells, but also cause damage and death in normal cells [5]. The problem is further complicated cancer, because most cases are found at an advanced stage, the survival rate is low, and costly to handle. Therefore it is necessary to do the discovery and development efforts of new cheaper anticancer.

One of the plants that attract attention is *C. gigantea*. It is a plant that originated in India and spread throughout tropical and subtropical regions of Africa and Asia. In ethnobotany leaves of *C. gigantea* believed to treat itching, trachoma, cough, constipation, and scabies; its flower is used as an asthma medication, nausea, and abdominal pain. While the latex is used to cure carbuncle, ekzema, toothache, syphilis, inflammation of the child’s ears, dysentery and swelling. The roots of this plant are used to treat gastric cancer. However inventarization of these plants is still low, so that the cytotoxic activity of *C. gigantea* in particular, which grows in the region of Aceh is not known yet. Especially regarding to its chemical content. Previous studies conducted by⁵ have isolated an anticancer compound pregnanon that is calotropon of ethanol extract of the roots of *C. gigantea*. *C. gigantea* plants have chemical constituents such as kardenolida, cardiac glycosides, flavonoids, pregnan, gigantisin and non-protein amino acids. During the screening of cytotoxic materials from tropical medicinal plants, the ethanol extract of the roots of *C. gigantea* showed cytotoxic activity against K562 chronic myelogenous leukemia, and human gastric cancer SGC-7901 in vitro using the MTT method with IC⁵₀ value of 9.7 mg/mL and 6.7 mg/mL. Bioassay-guided fractionation of the ethanol extract of *C. gigantea* roots produces a new compounds pregnanon, namely calotropon (1), with cardiac glycosides compounds (2). The structures of these compounds were determined by using 1D and 2D spectral data of NMR Spectroscopy. Compounds 1 and 2 showed significant cytotoxic activity against K562 cells and SGC-7901.
Methanol extract of the roots of this plant are used as larvical for *Tribolium castaneum* [7]. This plant has the same family with the plant *Calotropis procera* (Asclepiadaceae) which has been studied that the latex at a dose of 1000 mg/mL can kill 100% third instar larvae of *Aedes aegypti* in 24 hours [8], which showed that the latex of this plant is very toxic. The results of screening anticancer of ethyl acetate extracts of stem bark and leaves of *C. gigantea* with Brine Shrimp Lethality Test (BLST) method obtained a strong cytotoxic activity with LC$_{50}$ values of each: 39.73 ppm and 35.86 ppm [9]. Further research has been carried out fractionation of the ethyl acetate extract of the stem bark, phytochemical test and cytotoxicity test of combined fractions with the MTT method against P388 cells.

**MATERIALS AND METHODS**

**Collection of Plant Material**

*C. gigantea* was collected from the wild growing population in Keudee Aceh village, Kecamatan Banda Sakti, Kota Lhokseumawe during March 2014. The plant was identified in the Herbarium Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Syiah Kuala University. The collected plant parts (stem barks) were separated from undesirable materials. The plant parts were sliced into small pieces and dried in open air under shade for one week.

**The Extraction Process**

The dried stem bark of *C. gigantea* is taken as much as ± 2 kg. Furthermore, the bark is macerated with ethyl acetate solvent for 3x24 hours in order to obtain the extract solution. Maceration is repeated until the extracts obtained were almost clear. Then, the process followed by filtering and the filtrate was concentrated by using a vacuum rotary evaporator to produce concentrated ethyl acetate extract. Then, the weight of the extract is measured.

**Fractionation of Concentrated Ethyl Acetate Extract**

Concentrated ethyl acetate extract eluent system specified with a suitable solvent comparison using a TLC plate. Subsequently, the sample was drawn as much as 10 grams and its components are separated using a Vacuum Liquid Chromatography (VLC). Stationary phase in the form of silica gel, i.e Silica gel 60 G which is as much as 100 grams and a mobile phase of dichloromethane and methanol with gradient elution (based on the results of analysis by TLC). Accommodated fraction out of each 50 mL in erlenmeyer. The fractions were combined according to similarity of stain patterns after eluted with eluent system obtained and also sprayed with reagent seric sulfate. This is called the combined fractions. Then, the concentrated extracts and combined fractions are tested to identify chemical constituents and MTT assay to P388 cells.

**Qualitative phytochemical screening**

Chemical tests were performed on the ethyl acetate extract of stem bark of *C. gigantea* and its combined fractions using standard procedure to identify the phytoconstituens [10].

**MTT Assay**

Cytotoxicity assay was performed as the method that has been reported earlier [11,12]. The cells were harvested (2.5-3.0 x 104 cells/well) and inoculated on plates consisting of 96 wells. The cells were washed with PBS (phosphate buffered saline) and then inoculated cultured with and without sample (1 mg/mL of ethyl acetate extract from the stem bark of *C. gigantea* and combined fractions of ethyl acetate extract). After incubation for 72 h, the medium aspirated. 10 mL solution of MTT (5 mg/mL in PBS pH 7.2) was added to each well and the plate was incubated for 4 h at 37° C. After incubation, 100 mL of DMSO (<0.5%) was added to each well and then homogenized with a shaker for a color formazan stabilize for 15 minutes. Absorbance reading is using microplate reader at λ540 nm and the fraction of surviving cells was calculated. Artonin E (100 mg) was used as a reference drug. Inhibition of the cell is calculated as follows:

\[
\text{% Cytotoxicity} = \left(1 - \frac{\text{Abs test}}{\text{Abs control}}\right) \times 100
\]
RESULT AND DISCUSSION

Maceration of the dried stem bark of C. gigantea in ethyl acetate yielded a brownish green extract. Fractionation of the extract by VLC on silica gel gave 14 fractions (FIGURE 1). From TLC analysis, according to similarity of stain patterns, the fractions were combined into five combinations which were fraction A (fraction 1-3), fraction B (fraction 4), fraction C (fraction 5-10), fraction D (fraction 11-12) and fraction E (fraction 13-14).

FIGURE 1. The chromatogram of TLC from separated fractions after VLC.

Phytochemical compounds were screened in the ethyl acetate extracts of stem bark of C. gigantea and its fractions through qualitative method. The results indicated the presence of alkaloids, saponins, phenols, steroids, flavonoids, coumarins and the absence of terpenoids in the extract. From the fraction A-E showed that the steroid, flavonoids, phenolic, and coumarins were concentrated in the fraction B, while the alkaloids, steroids, saponins and coumarins were found in the fraction C, the coumarins were concentrated in the fraction A. Then, the fraction D contains saponins and significant quantities of steroids, while the fraction E contains only the saponin which was shown in table 1.

TABLE 1. Phytochemical screening of ethyl acetate extract of stem bark of C. gigantea and its combined fractions

<table>
<thead>
<tr>
<th>No.</th>
<th>Secondary Metabolites</th>
<th>Ethyl Acetate Extract</th>
<th>Combined Fractions</th>
<th>Observation Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Meyer's reagent</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Dragendoff's reagent</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Wagner's reagent</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Steroids</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Terpenoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Coumarins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Phenols</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+: presence; - : absence

TABLE (2). MTT assay of ethyl acetate extract of C. gigantean stem bark and its combined fractions
<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ethyl acetate extract</td>
<td>57.05</td>
</tr>
<tr>
<td>2.</td>
<td>Fraction A</td>
<td>60.37</td>
</tr>
<tr>
<td>3.</td>
<td>Fraction B</td>
<td>55.65</td>
</tr>
<tr>
<td>4.</td>
<td>Fraction C</td>
<td>58.26</td>
</tr>
<tr>
<td>5.</td>
<td>Fraction D</td>
<td>&gt;100</td>
</tr>
<tr>
<td>6.</td>
<td>Fraction E</td>
<td>&gt;100</td>
</tr>
<tr>
<td>7.</td>
<td>Artonin E (kontrol +)</td>
<td>0.6</td>
</tr>
</tbody>
</table>

The results from MTT assay indicated that the ethyl acetate extract and fraction A, B, and C have cytotoxic activity against murine leukemia P388 cells with value IC<sub>50</sub> as follow 57.05 µg/mL, 60.37 µg/mL, 55.65 µg/mL, and 58.26 µg/mL which was shown in Table 2, while fractions D and E less active. The Fraction B is most toxic to cancer cells in vitro compare to the other fractions, it might be phytochemicals analysis revealed the presence secondary metabolites has the most prominent amount in the fraction B. Phytochemical screening revealed the presence of various chemical constituents, which posses strong antioxidant activities. The antioxidant may prevent and cure cancer and other diseases by protecting the cells from damage caused by free radicals-the highly reactive oxygen compounds [13,14].

CONCLUSION

The phytochemical analysis revealed the bioactive metabolites which are responsible for the cytotoxicity in the ethyl acetate extract of C. gigantea stem bark and three fractions A, B and C against P388 cells. MTT assay results from the ethyl acetate extract and five combined fractions against P388 cancer cells is found that the ethyl acetate extract and three fractions combined (A, B and C) have cytotoxic activity with IC<sub>50</sub> value was 57.05 µg/mL, and fractions A, B, and C are as follows: 60.37, 55.65 and 58.26 µg/mL. Further study is required to isolate the lead compound responsible for this activity and to investigate cytotoxic activity to P388 cell lines for the development of new anticancer drug.

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REFERENCES