Exploration of the components of ethanol extract of herbal medicine origin of Papua Province-Indonesia that interact with the DNA of Escherichia coli

Rosye H. R. Tanjung¹ and Yohanis Ngili²

¹Department of Biology, Faculty of Mathematics and Natural Sciences, University of Cenderawasih, Jayapura-Indonesia
²Biochemistry Research Group, Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Cenderawasih, Jayapura-Indonesia

ABSTRACT

This research objective to investigate the interaction between the bacterium E. coli with the components of the ethanol extract of herbs as a chemical library (a collection of various chemical compounds) on the nature of biological materials in the province of Papua. E. coli as a target is one of the model organisms used in new drug development (drug discovery) in this study. This model is also consistent with the research of the human genome in an effort to balance the new strategy in the diagnosis, treatment, and prevention of disease. The interaction was investigated by growing the bacteria on solid media containing ethanol extracts of herbs, herbal ethanol extract encounters with bacterial chromosomal DNA by dot blotting method and the results observed by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). On the growth of bacteria on solid media containing fungi, bacteria continue to grow so that interaction can not be observed directly, while the method of TLC increased value retention factor (Rf) of ethanol extract of herbs before and after the interaction with DNA. For the eluent composition of methanol : water (6:4), the ethanol extract of herbs that have not diinteraksikan have Rf of 0.84 increased to 0.94 after DNA interactions. The results of the HPLC analysis provides more detailed information about the interactions that occurred with a reduction in height and broad peak at a retention time of 2.67; 12.8; and 16.9 min. This most likely indicates an interaction between the molecules of DNA with the compounds contained in the ethanol extracts of herbs.

Keywords: E. coli, ethanol extracts of herbs, TLC, HPLC, and Papua province of Indonesia

INTRODUCTION

Research in the field of drug discovery today continues to evolve in line with the growing need for drugs. It was known a lot of time, effort, and cost required to develop a new drug [1]. Hundreds of chemical compounds to be discovered and synthesized as well as an assortment of activity of these compounds to be studied. Therefore, many efforts were made to facilitate the things to be done in this new drug discovery research [2].

Drug discovery begins by determining a compound that has a specific biological activity. To achieve this, research is usually performed with encounters a collection of chemical compounds (chemical library) on a selected target molecules and eventually obtained a chemical component in the library that has a specific activity. Today there is a new phenomenon to combine the two major forces in front of us is the source of wealth of biodiversity that exists in nature Indonesia and the results of genome research world be it the human genome, plant genome, and the genome of the microorganism. Biological natural resources can be used as a chemical library that will be obtained by a component of the natural resources that can be used in drug development [3-4]. Sources of biological nature can be exploited through a simple chemical process that is extraction. The use of the wealth of natural materials is very advantageous considering Indonesia is naturally blessed with diverse natural resources are very rich.
To detect the presence or absence of interaction between the target molecule with chemical library is usually used molecular screening methods. One of the screening methods used in drug discovery research is the High Throughput Screening (HTS). HTS is an instrument with cutting edge technology that can detect the molecular level interactions between a chemical compound with the target molecule. Methods of screening using this tool can be done on a large scale because of the HTS has the advantage to screen at very high speeds, which can detect a sample of up to 1,000 samples per week even more. However, this HTS methods also have disadvantages, namely a long process and thus also takes a long time to arrive at the stage of finding compounds that interact and also expensive.

From some of these weaknesses, the this research aims to find an alternative method of detecting interaction between a chemical compound with a target molecule. The development of this method is expected to be generating a more effective method by phasing procedure simpler. As a chemical library provides a collection of chemical compounds that will be used to detect interactions traditional Indonesian herbal medicine. Selection of herbal medicine as a chemical library for herbal medicine itself is a result of extraction of natural wealth of Indonesia and has been known to useful by those who use it. Wealth of natural materials when utilized optimally will contribute significantly to human interests. Moreover, because herbal medicine has been used as a drug it is predicted that these herbs contain components that have a specific activity that can be detected with this alternative method [5].

In this study the DNA of *Escherichia coli* was used as a model for the target molecule. *E. coli* bacteria are generally known to be harmful organisms in the human intestine. Besides *E. coli* is also a type of bacteria that is cultured in laboratory conditions until will facilitate the research process. The number of research on this bacterium *E. coli* into an organism makes the most understandable. Another reason is that it is often used as a model in the drug discovery process as *E. coli* DNA that are similar to human DNA.

**MATERIALS AND METHODS**

**Culturing the bacterium *Escherichia coli***
The medium used for culturing the bacteria in this study is the media *Luria Bertani* (LB) solid and liquid. LB liquid medium is a mixture of bactotryptone 1% (w/v), yeast extract 0.5% (w/v) and NaCl 1% (w/v) dissolved in distilled water and then sterilized using an autoclave. For the manufacture of solid media, materials and compositions used together with the liquid media but added *bactoagar* 1.5% (w/v). Once sterilized if necessary added ampicillin, *ampicillin* addition of 50 µg/ml, 50 mL of media is done after the temperature dropped about 60 °C. Then the solid medium is poured into a petri dish and wait for it to freeze [6].

Culturing on solid media is done by taking a single bacterial colony of the bacteria *Escherichia coli* seedlings using the control loop and then streaked onto solid media recently. After it was incubated at 37 °C for 16-18 h without shaking. Culturing in a liquid medium is done in the same way, namely by moving a colony of bacteria from solid media into new media with the rods ose liquid and then incubated at 30 °C for 16-18 h or overnight with shaking using a shaker to obtain freshly prepared culture isolated DNA [6].

**Analysis of the effect of the ethanol extract of herb against bacteria *E. coli* by culturing in solid media**
For this purpose provided two Petri dishes containing solid LB medium for the growth of the bacteria *E. coli*. In the petri dish first to contain solid media dripped ethanol extracts of herbs 25% (w/v) of 40 mL. and then spread until evenly across the surface in order to use rod L, so that the media in a petri dish both of which were not added extracts of herbs because it will be used as control.

In both solid media is then grown bacteria *E. coli* JM109. After that both of them were incubated for 16-18 h at a temperature of 37 °C without shaking to see if the *E. coli* bacteria can still grow on media that has been given the herbal extract [6].

**Interaction of ethanol extract of herb with the bacteria *E. coli* DNA**
Interaction the ethanol extract with DNA done by *dot blotting*. Initially prepared nylon membrane *Hybond-N*+ cut to size 1x1 cm. DNA dropped on the membrane of *E. coli* at a concentration of 200 ng/ml and then dried in the open air. DNA on the membrane and then irradiated with UV light for approximately 1 min. After that the membrane containing the DNA is placed on filter paper, each for five minutes, which previously had been dipped in the solutions, which are, respectively: 1.5 M NaCl, 0.5 M NaOH, 1 M Tris-Cl, 1.5 M NaCl, and finally with SSC 10x.

After that the membrane containing the DNA is incorporated into 400 µL of 25% ethanol extracts of herbs. To improve the interaction of ethanol extracts of herbs with DNA, membranes moist incubation is done with a waterbath and set at 37 °C for 16 h. After that the membrane containing the DNA was removed from the ethanol extract of herbs and herbal extracts that have interacted with the DNA is ready for further analysis. As is standard
for analyzing the interaction is prepared in the form of standards or controls 25% ethanol extracts of herbs were given the same treatment with the sample to be analyzed, but the membrane that is inserted does not contain DNA, but only in the form of empty membranes.

**The execution analysis by thin layer chromatography**

Analysis by thin layer chromatography (TLC) is performed with some variations eluent. Variations eluent used was methanol : water with a ratio of 6 : 4 (w/v), hexane : ethyl acetate in a ratio of 1 : 1 (w/v), chloroform : methanol with a ratio of 9 : 1 (w/v), and last, chloroform : methanol in the ratio 1 : 1 (w/v). For the purposes of this thin layer chromatography prepared silk gel thin plate that cut the size of 9x1 cm. Then given upper and lower limits so that the course of eluent as high as 8 cm. In the silica plate sample spotted ethanol extracts of herbs after interaction with DNA and also standardized herbal extracts ethanol without any interaction with DNA. The plates are then put in the eluent in the chamber and wait until the eluent rises to a specified threshold. The same thing done for each variation eluent [7-8].

**The execution of analysis by high performance liquid chromatography (HPLC)**

Execution of the HPLC analysis is quite simple: by using HPLC column type used are C<sub>18</sub> columns with a length of 15 cm. Wavelength measurement time is 254 nm and the flow rate was adjusted to 1 ml/min [7-8].

Eluent used in this analysis is a variation of eluent methanol : water with a ratio of 6 : 4 (v/v). Before use eluent is inserted first into the ultrasonic bath to improve the quality of the eluent them to precipitate impurities that may be present in the eluent. The analysis was performed by injecting samples of the ethanol extract of herbs on HPLC instrument. Then from the HPLC apparatus will be printed chromatogram of the separation of the sample is injected. The same thing has been done to a standard herbal extracts without any interaction with DNA in order to obtain two chromatograms, standards and samples, which will then be compared to analyze the interactions happened.

**RESULTS AND DISCUSSION**

Research on the interaction between the bacterium E. coli with the ethanol extract of herbs is the first step to determine the effect of herbal medicine against E. coli bacteria in supporting drug discovery research. This study, together with the research of the human genome, is expected to pave the way for the discovery of new strategies in the diagnosis, treatment, and prevention of diseases particularly associated with the bacteria E. coli. In the results and discussion will analyze the results obtained in the study of the interaction of E. coli bacteria with the ethanol extract of herbs.

**Culturing the bacteria E. coli**

E. coli bacteria breeding purposes is to provide stock cultures and cell cultures were prepared freshly isolated. Breeding is done in solid and liquid media. Seed scratched bacteria on solid medium and incubated at 37 °C for 14-16 h. For culturing in a liquid medium, the bacteria are taken from the solid media stock to the liquid medium and incubated with agitation at a temperature and time of incubation of the same. Liquid medium turns into a murky due to the growth of bacteria in it.

Bacterial growth media consists of a mixture of tryptone, yeast extract, and NaCl and added that for solid media. Media with the composition as it is a medium Luria Bertani (LB) which is a complex media to the identity and quantity of its components is not known precisely. To study the growth and culturing, a chemical compound commonly used for culturing liquid medium is a solution of substances from natural sources such as yeast extract, beef extract, and milk, which composition is not known completely and accurately.

On solid media typically added in order, a complex carbohydrate (galactose) obtained from certain marine algae. To be used only as a compactor but do not provide nutrients for microorganisms. On LB media, tryptone is a source of amino acids and peptides while a source nirtogen yeast extract, sugar, and organic and inorganic nutrients [9].

Bacteria in solid media is used as stock cultures for storage. These bacteria must be regenerated every three to four weeks to provide a new source of nutrients for the bacteria. Growth in liquid media aiming to produce cultured fresh (fresh overnight culture) for the isolation of chromosomal DNA.

**Isolation of chromosomal DNA of E. coli**

Isolation of DNA is intended to get the chromosomal DNA of E. coli bacteria can be studied interactions with herbal medicine as a chemical library on the next process. In the process of isolation of chromosomal DNA, was first centrifuge fresh cultures of bacteria and taken pellets, then resuspended in lysis buffer Tris-HCl, pH 8.0 containing EDTA, lysozyme, and sucrose and incubated 30 min at 37 °C. The cell suspension happened then added a solution
of NaCl containing 1% SDS, and proteinase K 2.6 mg/mL and incubated at 37 °C for 60 min. After incubation the reaction mixture was added 3 M sodium acetate pH 5.2 and cold absolute ethanol, then centrifugation back at 4 °C. After added 70% cold alcohol on the DNA pellet, the solution was recentrifuged. The resulting pellets resuspended with TE solution pH 8.0.

Isolation of DNA generally consist of cell isolation, lysis of cells to release their contents, removal of protein from the cell extract, and concentrating DNA. Centrifugation fresh culture is the stage of isolation cells because the cells have been isolated is then degraded with lysis buffer Tris-HCl pH 8.0 containing EDTA, lysozyme, and sucrose so that the cell wall and membrane of bacterial cells damaged. EDTA acts as a *chelating agent* that is clamped magnesium ions so that the DNA is free of magnesium ion (or other divalent cations), which is a cofactor in maintaining overall sheath cells. While lysozyme is an enzyme that can cut polymeric compounds cause cell wall stiffness. Sucrose is added to prevent the outbreak of rapid cell. Sodium dodecyl sulphate (SDS) is added to remove the lipid molecules in the cell membrane that can damage cell membranes and helps the whole process of lysis is performed. SDS is a kind of detergent to remove the layer of fat. The addition of proteinase K is to degrade proteins on the cell suspension, because K protein is an enzyme that can break down protein molecules. DNA concentration is done by adding sodium acetate pH 5.2 and cold absolute ethanol which has been concentrated DNA was washed with cold 70% alcohol. For the storage of DNA, the DNA pellet resuspended with TE pH 8.0. The existence of DNA was tested by agarose gel electrophoresis.

**Electrophoresis using an agarose gel**

Electrophoresis was aimed at testing the isolated chromosome. Agarose gel containing EtBr prepared and DNA isolated ddH₂O along with loading buffer and gel inserted in the well that has been dipped in 1x TAE buffer. λ DNA are cut with *Hind*III was used as a standard. 80 volt electricity flowing in the gel. After irradiation by ultraviolet rays obtained results show the DNA bands.

DNA molecules, such as proteins and other biological compounds carry an electric charge. DNA molecules are electrically charged negatively, so that DNA molecules can migrate to the negative pole to the positive pole, when the DNA molecule placed in an electric field. The addition of ddH₂O is as a solvent. EtBr gel can interact with the DNA so that the ultraviolet irradiation, DNA bands will glow.

λ DNA are cut with *Hind*III commonly used as a standard because it has fragments known for certain size so that it can be used as a comparison to the DNA produced. DNA bands which happen to show their chromosomal DNA is isolated so that DNA can be used for further processing.

**Planting bacteria on solid media containing herbal medicine**

These experiments are preliminary experiments in the study of interaction of DNA with herbs. In this experiment the bacteria *E. coli* was grown on solid media containing herbs. For that performed various concentrations and variations in the volume of ethanol extracts of herbs to find the optimum concentration of both volume and interaction of DNA with herbs. For this purpose use three varying concentrations of ethanol extracts of herbs: 25% (w/v), 50% (w/v), and 75% (w/v).

For each concentration done some variation of volume: 20 mL, 40 mL, 60 mL, 100 mL and 300 mL. In this experiment, it takes 18 solid medium that contains herbs along with two other solid media that contains no herbal medicine as a positive control and a negative control. On solid media as negative, is used as an antibiotic ampicillin. The addition of herbs on solid media is also done in three ways: (1) leveling herbs directly on solid media LB, (2) incorporate herbs into solid media before the media becomes solid, and (3) make herbal medicine as the top order in on solid media. *E. coli* bacteria grown on solid media overall, after incubated at 37 °C for 16 h seen the results obtained. In all solid media containing herbs, bacteria keep growing. On solid media as a positive control, the bacteria *E. coli* was grown. While on solid media containing ampicillin (negative control) bacteria can not grow.

Drugs included in the growth medium of bacteria usually inhibit the synthesis of DNA, RNA, and proteins such bacteria thus indirectly inhibit the growth of bacteria. This phenomenon is seen in the negative control experiments are on solid media containing ampicillin, *E. coli* bacteria can not grow. Sometimes bacterial growth is not hampered due to the synthesis of DNA, RNA, and proteins are not hampered by a lack of drugs or bacteria is furnished by certain elements that can build up resistance to certain drugs. For example, *E. coli* containing pUC19 be resistant to the antibiotic ampicillin. This happens because ampicillin may bind to and inhibit enzymes in the membrane of bacteria involved in bacterial cell wall synthesis.

Herbal medicine as a chemical compound derived from natural sources, should also be able to influence the growth of bacteria. Herbal medicine as a *chemical library* (a collection of various kinds of chemical compounds) is also
likely to contain components of chemical compounds inhibiting the growth of bacteria, such as ampicillin. The components of herbs so complex that most likely also contain a compound herbal enzyme β-lactamase that can inactivate bacterial growth inhibiting components in herbal medicine. Effect of the antagonist resulted in bacteria continued to grow in media containing herbs. In other words, no herbal extract can kill these bacteria. Nevertheless, the interaction between the DNA of *E. coli* bacteria with the ethanol extract of herbs will most likely stay there, but can not be seen directly through the growth of bacteria. The bacteria may continue to grow, although the growth media are still added ethanol extracts of herbs and even to a volume of 300 mL and a concentration of 75% (w/v), but maybe the interaction of DNA with ethanol extract of herbs will be different than if the addition of ethanol extract of herbs at a concentration of 25% (w/v) and 20 mL volume alone. This is what needs to be further investigated with other methods that provide more detailed information about the interaction of *E. coli* bacterial DNA with ethanol extracts of herbs.

**Interaction the ethanol extract with *E. coli* DNA through the membrane**

In this research, the *dot blotting* method is used to reacting with the DNA sample to obtain medicinal herbs that have interacted with DNA. The experiment starts with a drop of DNA on Hybond-N+ membrane (Amersham) and irradiated with ultraviolet light to alter the conformation of other chemical compounds. Additionally, empty membrane without DNA was also treated similarly to be used as a standard. After the two membranes were placed on filter paper containing 0.5 M NaOH with the aim to denature the DNA. After the two membranes are placed on top of the filter that has been dipped in a solution of 1 M Tris-Cl and 1.5 M NaCl to neutralize the solutions of NaCl and NaOH so it does not react further. Further washing with a solution of 10x SSC in the same way, and then incubated overnight to enhance the reaction between DNA and herbs. The results are then analyzed in two ways: *Thin Layer Chromatography* (TLC) method and *High Performance Liquid Chromatography* (HPLC) method.

**RESULTS AND DISCUSSION**

The following describes important aspects related to the results of research that has been done in the analysis process of interaction between the ethanol extract of herbs and *E. coli* bacteria.

**Analysis using thin layer chromatography**

The experiment begins with a silica samples of herbal extracts that have been treated with standard DNA and herbs without DNA in the cell plate silica, then eluted with certain eluent composition.

The experiments were performed by varying the composition of the eluent to obtain the proper eluent composition to observe the interactions between DNA with ethanol extract of herbs. The most well eluent composition will make a point of sampling the plates move half between the standard of the upper limit of the eluent (Rf value = 0.5). If the stain does not migrate properly and is located close to the point of initial stain more polar eluent should be used.

On the composition of the first eluent is methanol : water (6:4), the sample solution and the standards of the most up and separated into two spots, but some are still lagging below. Similarly happened to the second eluent composition, namely hexane : ethyl acetate (1:1). The difference is in the composition of the eluent of the first, the price difference Rf (retention factor) between standard samples and large enough to be observed is 0.94 for samples to 0.84 for standards. Because Rf sample is larger than the standard means Rf sample migrate farther than the standards (the ethanol extract of herbs which are not reacted with DNA). On the composition of the second eluent, Rf standards and samples are not much different to a standard that is 0.41 and 0.42 for the sample. Samples migrate faster than the standard. In the third and fourth eluent composition is chloroform: water (1:1) and chloroform : methanol (9:1) most of the standard sample solution and migrate upward. On the fourth composition, still lagging a little stain underneath. Observations Rf hard to do in two compositions, because the results are not so clear and the distance difference is hardly noticeable stains.

Thin-layer chromatography which carried out aimed at observing the differences in polarity of herbs before reacted with DNA and herbs that have been reacted with the DNA to see the difference in distance between the two stains. If it was such a case, means that there are changes in the nature due to the effect of the interaction between the herbal extract and DNA.

The experimental results of thin layer chromatography generally showed the addition of stains distance between herbs before reacted with the DNA so that it can be concluded that there have been interactions between herbal medicine with DNA molecules. To see the interactions between DNA with ethanol extract of herbs more clearly analyzed using a high performance liquid chromatography (HPLC).
Analysis using high performance liquid chromatography method

In the content analysis, and sample standard herbal extracts injected into the HPLC instrument and run using the eluent methanol : water (6:4). For the results obtained by the two chromatograms as seemingly in the picture below (Fig. 1 and 2).

**Fig 1.** Standard HPLC chromatograms of herbal extracts after interacting with DNA. This can be seen on the chromatogram separation of the components of herbal extracts in more detail, so that the interaction of DNA with herbs can be observed.

**Fig 2.** HPLC chromatograms of the herbal extract samples after interacting with DNA. In this chromatogram peaks there are some changes when compared to the standard chromatograms.

Both profiles chromatogram obtained will be compared to see the difference between the two peaks of the chromatogram. This comparison is done because the initial hypothesis of this study is that the process of interaction through the dot blotting method is expected to be an interaction that causes changes herbal extracts chromatogram profile before and after the interaction of the interaction. With the interaction, the peaks in the chromatogram which...
is a representation of the components in the ethanol extract of herbs will turn high and wide peak. The change in height and peak area is due to the local extracts early (before the interaction process) which has lost some of its components.

Based on the results obtained in the second image (Fig. 2) above shows that the change in the chromatogram peaks are generally similar to each other. There is a chromatogram peaks that are changing height and peak area. To be able to see both of these changes, carried out extensive calculations strategy peaks in the chromatogram standard and sample. Peak area which counted on HPLC instruments are converted so that obtained in Table 1. The highlight standard chromatogram taken as the first standard and given a value of 100 as a benchmark for other peaks are converted via a formula b/a x 100 with a broad peak is the first standard by a value of 100, and b is the peak area of components to be converted (second standard peak). This is done also to the sample. Table 1 presented the first comprehensive value comparison conducted on the peak retention time of 1.2 min, which is the first standard solvent peak. It is seen that peaks with retention of 2.67; 12.8; and 16.9 min dropped drastically while others are only changed slightly. Making the retention time of 1.2 min as a benchmark based on the assumption that this peak is the peak of the changes are not very significant, because the solvent in HPLC analysis relative has high stability that will not change even if there is a process of interaction in the HPLC analysis.

Table 1. Comprehensive initial comparison value peaks. Peak with a retention time of 1.2 as the first standard, the peaks with retention time of 2.6; 12.8; and 16.9 underwent significant changes

<table>
<thead>
<tr>
<th>Retention time</th>
<th>Peak area</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>Sample</td>
<td>Standard</td>
</tr>
<tr>
<td>0.962</td>
<td>0.995</td>
<td>112247720</td>
</tr>
<tr>
<td>1.262*</td>
<td>1.290*</td>
<td>11613489</td>
</tr>
<tr>
<td>1.852</td>
<td>1.804</td>
<td>34563552</td>
</tr>
<tr>
<td>2.196</td>
<td>2.231</td>
<td>15077424</td>
</tr>
<tr>
<td>2.67</td>
<td>2.707</td>
<td>95745856</td>
</tr>
<tr>
<td>3.241</td>
<td>3.294</td>
<td>314248</td>
</tr>
<tr>
<td>4.02</td>
<td>4.265</td>
<td>2064862</td>
</tr>
<tr>
<td>4.905</td>
<td>4.981</td>
<td>6422323</td>
</tr>
<tr>
<td>5.577</td>
<td>5.636</td>
<td>18187360</td>
</tr>
<tr>
<td>7.535</td>
<td>7.640</td>
<td>3467930</td>
</tr>
<tr>
<td>9.776</td>
<td>9.960</td>
<td>6174147</td>
</tr>
<tr>
<td>10.434</td>
<td>10.709</td>
<td>5823491</td>
</tr>
<tr>
<td>12.813</td>
<td>13.110</td>
<td>38715616</td>
</tr>
<tr>
<td>16.926</td>
<td>17.294</td>
<td>35210624</td>
</tr>
</tbody>
</table>

* The first standard is taken from one of the components of herbal medicine
* Peak is regarded as significant changes

Table 2 is the comparison of two broad peaks. The second comparison is done to support the results of the first comparison. Calculations on the second comparison is done by taking the herbal components which peaks slightly changed, namely the peak with retention time of 4.02. Visible results are consistent among the first comparison results in Table 1 with the results of the second comparison in Table 2.

Table 2. The second comparison value for broad peaks. The first peak is taken from the peak with a retention time of 4.02. Changes that occur consistently at the top with a retention time of 2.6; 12.8; and 16.9

<table>
<thead>
<tr>
<th>Retention time</th>
<th>Peak area</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>Sample</td>
<td>Standard</td>
</tr>
<tr>
<td>0.962</td>
<td>0.995</td>
<td>112247720</td>
</tr>
<tr>
<td>1.262</td>
<td>1.290</td>
<td>11613489</td>
</tr>
<tr>
<td>1.852</td>
<td>1.804</td>
<td>34563552</td>
</tr>
<tr>
<td>2.196</td>
<td>2.231</td>
<td>15077424</td>
</tr>
<tr>
<td>2.67</td>
<td>2.707</td>
<td>95745856</td>
</tr>
<tr>
<td>3.241</td>
<td>3.294</td>
<td>314248</td>
</tr>
<tr>
<td>4.02*</td>
<td>4.265*</td>
<td>2064862</td>
</tr>
<tr>
<td>4.905</td>
<td>4.981</td>
<td>6422323</td>
</tr>
<tr>
<td>5.577</td>
<td>5.636</td>
<td>18187360</td>
</tr>
<tr>
<td>7.535</td>
<td>7.640</td>
<td>3467930</td>
</tr>
<tr>
<td>9.776</td>
<td>9.960</td>
<td>6174147</td>
</tr>
<tr>
<td>10.434</td>
<td>10.709</td>
<td>5823491</td>
</tr>
<tr>
<td>12.813</td>
<td>13.110</td>
<td>38715616</td>
</tr>
<tr>
<td>16.926</td>
<td>17.294</td>
<td>35210624</td>
</tr>
</tbody>
</table>

* The first standard is taken from one of the components of herbal medicine
* Peak is regarded as significant changes
There are many different types of interactions that can occur at the time of analysis by HPLC performed [10-12], namely:
1. The first type: the interaction of a component in ethanol extracts of herbs, for example X components that interact only with the membrane.
2. The second type: the interaction components x with the membrane DNA.
3. The third type: the interaction of components x only with DNA
4. The fourth type: the interaction of the component x of the membrane and component A in DNA. So there are two different components in herbal extracts that interact each membranes and DNA.

Actually it would be many other types of interactions that can occur in this analysis, but all four types had quite elaborate ideas about the possibilities of interaction. In this study, the results are more focused on the type of the third, but the sample on the chromatogram peak does not disappear completely, but reduced peak height alone. This means that only some components of herbs disappeared from the chromatogram profiles obtained samples. Based on these results, it can be concluded that the ethanol extract of herbs can interact with the DNA of E. coli. This interaction can be detected by TLC and HPLC methods. For further research can be done screening the second stage of looking for the kind of interaction happened and the potential biological activity of herbal medicine as a lead compound.

Various research in the world also involve biological materials result mainly of plants in various screening process as the search for new drugs (drug discovery). African crops such as C. sanguinolenta, S. gossweileri, and R. serpentine containing alkaloids Cryptolepine, Metadine, and Serpentine potential as a cancer drug. Research on these alkaloids is done through observation of interactions with human DNA and their effect on topoisomerase II, an enzyme that plays a role in DNA replication process.

Results showed that DNA and topoisomerase II is the major target of three alkaloids earlier. By using leukemia cells HL60, investigated the effect of mobile due to the inhibition of topoisomerase II by Cryptolepine. There is no change in the distribution of the cell cycle as a whole and there is no process of apoptosis caused by the activity of this alkaloid.

Natural products has been providing comprehensive and rich source for new drug discovery research. This natural resource has been proven to have potential applications in the treatment of diseases that attack humans contemporary. More than half a century, cancer chemotherapy have involved natural products such as antibiotics produced Streptomyces (including bleomycin, actinomycin, and daunomycin) and drugs from plants such as vincristine, etoposide, and so on. In addition to cancer, other diseases may also be cured with medicinal plants. these are the things which lead research natural resources better. Herbal medicine as chemical library is a source of potential eligible to be further investigated, especially the interaction and biological activity against the target.

Target molecules such as DNA has proven instrumental in testing biological processes associated with diseases, such as research on antitumor antibiotic drug which forms a covalent interactions of DNA at the 5’carbon its deoxyribose. In lupus disease research, human diseases associated with the immune system obtained information that a particular nucleotide sequence in the ssDNA antigens can be recognized by anti-ssDNA antibodies. This research needs to be done to study the interactions that occur in the process of introduction of an antigen by the antibody. Besides the interaction of DNA with proteins involved in disease therapy process widely studied. This research includes interaction in the process of inhibition of transcription factors and processes of complex formation in the process of transcription. This is what led to the study of the interaction of DNA is very important to do. Interaction of ethanol extract of herbs with DNA of E. coli which has proven its existence with TLC and HPLC methods are expected to provide an initial contribution to the further testing of the new drug discovery research [1,7,8]. Further research may be directed to analysis at the interaction of mutations with diseases caused [13-16].

CONCLUSION

This research objective to understand the interaction between E. coli as a target by the ethanol extract of herbs as a chemical library. The results show the interaction between the two interactions happened can be observed at a molecular level with a method which cheap, fast, and simple with TLC and HPLC methods. HPLC method provides more detailed information about the interactions that occur over TLC method. In the HPLC method visible components which likely to interact with the DNA of the bacteria E. coli. This is demonstrated through the peaks at a retention time of 2.67; 12.8; and 16.9 min which significantly changed.
Acknowledgements
Authors gratefully acknowledge the financial support of this work by Directorat Research and Community Services (Ditlitabmas). Thanks to Chairman of Biochemistry Laboratory for research facility during the research process.

REFERENCES