No. Reg : 191050000011745

PROPOSAL
SHORT COURSE OVERSEAS RESEARCH METHODOLOGY / BANTUAN EKSPOSE METODOLOGI RISET BERTANDAR INTERNASIONAL (BE-SCMRI)

JUDUL PROPOSAL :

ANTI BACTERIA PRODUCTION FROM VARIOUS TYPES OF FLIES WHICH INSPIRED FROM HADIS BUKHARY ABOUT FLIES (A MULTIYEARS INTEGRATED SCIENCE AND RELIGION RESEARCH)

Disusun oleh :

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DIREKTORAT PENDIDIKAN TINGGI KEAGAMAAN ISLAM
DIREKTORAT JENDERAL PENDIDIKAN ISLAM
KEMENTERIAN AGAMA RI
TAHUN 2019

I. Titles

Anti Bacteria Production from Various Types of Flies which Inspired from Hadis Bukhary about flies (a multiyears Integrated Science and Religion Research)

II. Background

قَالَ النَّبِيُّ صَلَّى اللَّهُ عَلَيْهِ وَسَلَّمَ إِذَا وَقَعَ الذُّبَابُ فِي شَرَابِ أَحَدِكُمْ فَلْيَغْمِسْهُ ثُمَّ لِيَنْزِعْهُ فَإِنَّ فِي إِحْدَى جَنَاحَيْهِ دَاءً وَالْأُخْرَى شِفَاءً.(رواه البخارى)

Meaning:

The Prophet. said: If there is a fly that falls on your drink then sink it then lift it, because on one wing the disease and the other wing there is the medicine[1]

Inspired by the hadis contained in the Sahih Bukhary no. 5336 above, two studies have been carried out in the biology department of UIN Alauddin Makassar in 2016, concerning the identification of biochemical, microbiological and molecular bacteria found on the left and right wing of house flies (*Musca Domestica*), and research on bacterial identification in biochemistry, microbiology and molecular found on the left and right wings of green flies (*Chrysomya sp*).

The fact that we obtained from the first study was that Bacillus Cereus bacteria were identified from the wings of the house fly (Musca Domestica). Bacillus Cereus bacteria are pathogenic bacteria that can cause disease in humans, these bacteria are able to produce spores that are resistant to heat and dehydration. Cases of poisoning that have occurred and have been reported to date are often associated with processed foods from vegetable flour such as pasta, rice, potatoes, bread and noodles.[2]

In the second study, the results of 3 types of bacteria in the wings of green flies (*Chrysomya sp*), namely Acinetobacter baumannii, Escherichia coli and Pantoea agglomerans. These 3 species of bacteria are pathogenic bacteria, which act as carrier vectors of a disease. That is the bacterium Acinetobacter baumannii, causing pneumonia, meningitis, septicemia, burns or surgical wounds. Escherichia coli causes acute diarrhea. And Pantoea agglomerans cause bacteremia, lower respiratory tract infections, skin infections, soft tissue

infections, urinary tract infections, endocarditis, intraabdominal infections, septic arthritis, osteomyelitis, and eye infections. [2]

From the two studies, then an inspired exploration study of the Bukhary hadith was conducted on fly so many pathogenic bacteria that can cause disease in humans, while on the one hand flies are very close to human life. Bukhary Hadith seems to be an enlightenment that behind the pathogenic bacteria found in flies, it turns out that Allah has entrusted anti-bacteria to the other wing of the fly. Scientifically this is very acceptable, given the fact that flies can live even with bacteria in their wings, meaning that the true designer has prepared anti-bacteria on the other wing. This then inspired us to do the Grand Design Multy Years Research which is broadly divided into:

1. The first stage (2019-2020): Identification of bacteria in the wings of flies in the residential area at the district Gowa sulsel (in addition to the *Musca Domestica* fly and *Chrysomya sp* fly that have been studied before).
2. Second stage (2020): Antagonistic test of pathogenic bacteria obtained from the first stage with various kinds of bacteria obtained from other wings of each flies.
3. The third stage (2021): Isolation, identification and purification of anti-bacterial compounds obtained from the second stage.

III. PROBLEM

1. What is the hadith contained in the Sahih Bukhary book no. 5336 can be implemented in a systematic science framework?
2. Can bacteria be identified in the wings of the flies at the residential area of Gowa sulsel district?
3. Can be done Antagonist test Pathogenic bacteria from fly wings against various kinds of bacteria obtained from other wings of each flies tudied?
4. Can anti-bacterial products be produced from wings of flies?

IV. GOAL

1. Implementation of the hadith contained in the Sahih Bukhary book no. 5336 in a systematic scientific framework.

2. Identifying the type of bacteria on the wing of the type of flies studied in the residential environment in Gowa sulsel district

3. Produce data Antagonistic test Pathogenic bacteria from fly wings against various kinds of bacteria obtained from other wings of each flies studied.

4. Produces anti-bacterial products from flies wings.

V. ANOTHER RESEARCH

1. Abu Salma, 2007 grew bacteria from the right and left wings of flies in two different petri dishes. In Petri dish 2, after being identified it was found that the media was overgrown with colonies of *Staphylococcus sp* which is the cause of various diseases. As for cup 1, growing microorganisms which after being identified are *Actinomyces* bacteria that produce antibiotics. These bacteria usually produce antibiotics that can be extracted, namely actinomycetin and actinomycin which functions to lyse bacteria and are antibacterial and antifungal. Similar results were obtained for other types of flies that contained pathogenic bacteria *Salmonella sp* and *Proteus sp*, which were inhibited by the growth of Actinomyces. The entry of flies in food or drink, without being dyed and dyed, turned out to give significant different results. The conclusion of Abu Salma's research is that this justifies what is said by the noble prophet Naballallhu, alaihi wa Sallam, that on the wings of the fly there is both disease and antidote. It is true that Allôh and the true are Rasūlullâh Shallâllâhu alaihi wa Sallam.

   It is different from Abu Salma's research that this research explores various types of flies found in the housing area of Gowa sulsel district.

2. Retno Hestiningsih (2004), *Chrysomya sp* fly was dominantly found in the final waste collection site ; Among the four estimated species of bacteria that cause gastrointestinal disease (*Eschericia. coli, Salmonella sp, Shigella sp,* and *Vibrio sp*) are not all found, only *E. coli* can be found, while other species that can be identified are *Klebsiella pnemoniae, Bacillus sp.* and *Enterobacter aerogenes* (dominant in flies and trash in the location of waste storage that is used as a sampling location), then *Staphylococcus aureus, Streptococcus sp., Proteus morgani* and *Proteusmirabilis*; Bacterial contamination in *Chrysomya megacephala* fly and *Musca domestica* fly comes from garbage shelters.

   The difference is that the location of the study is not in a garbage collection site.

VI. THEORITICAL

From the hadith of the Bukhary contained in the background there is a law that can be taken, namely that the fly is not unclean. Because, if the fly is unclean then the Prophet. will not tell to dip but tell to throw it away. But in this hadith does not emphasize whether the fly is unclean or not, but this hadith emphasizes more on the disease that exists in flies and the presence of drugs in the flies 'wings, so the Prophet ordered to dip the fly to remove the medicinal elements contained in the flies' wings.

Clasification of Green fly (*Chrysomya* sp.) as an example

Classification *Chrysomya* sp according to Kurahashi (Evenhuis 1989 in Soviana 1996) :

Kingdom : Animalia

Phylum : Arthropoda

Class : Hexapoda

Ordo : Diptera

Famili : Calliphoridae

Genus : Chrysomya

Spesies : *Chrysomya* sp.

Impact caused by fly

According to Ismail bin Jakarsi (2008), the impact that can be caused by fly,

a. Positive impact
   Like other insects, flies serve to decipher dead animals. Flies are also beneficial to other plants. Some species of flies are very instrumental in the pollination process. The role of fly is important in the process of decay of animals and plants in the environment, also in the process of pollination and other insect predators.
b. Negative impact
   Famous flies in the spread of several diseases such as dysentery, fever, and infectious and parasitic diseases, causes of myiasis, and vectors of several infectious diseases such as kala-azar and black fever sleeping sickness, and others.

General Review of Bacteria

Bacteria are one class of prokaryotic organisms (do not have a core sheath). Bacteria as living things certainly have genetic information in the form of DNA, but not localized in a special place (nucleus) and no core membrane. The form of bacterial DNA is circular, long and commonly called nucleoi. In bacteria DNA does not have introns and is only composed of axons. Bacteria also have extractromosomal DNA which is incorporated into a small and circular shaped plasmid (Jawetz, 2004).

The factors that influence bacterial growth are:

a. Energy source, which is needed for synthetic reactions that require energy in growth and restoration, maintenance of fluid balance, motion and so on.
b. Carbon source.
c. Source of nitrogen, mostly for the synthesis of proteins and nucleic acids.
d. Sources of inorganic salts, especially folate and sulfate as anions; and potassium, sodium magnesium, calcium, iron, manganese as cations.
e. Certain bacteria need additional growth factors, also called bacterial vitamins, in small amounts for essential metabolic synthesis (Irianto, 2006).

VII. COLLECTING DATA AND METHODE

STAGE I. Identify the type of bacteria in the wings of flies that are in the residential environment in the district Gowa sulsel

1. Sterilization of Tools

The tools that will be used are washed and then rinsed with distilled water, then the glass tools are sterilized using an autoclave at a temperature of 121 ^{0}C with a pressure of 2 atm for 2 hours. Metal tools are sterilized by means of being discharged using a spirit lamp with a pressure of 15 PSI temperature of 121 C

2. Sample Preparation

The samples used are wings of various types of flies. Sampling was carried out in a sterile way.

3. Making a sample suspension

The fly wing is inserted into an eppendorf tube containing PBS. The purpose of this technique in principle is to dissolve or release microbes from the substrate.

4. Making Media

The materials to be used are prepared for the manufacture of each medium. Making media used for planting bacteria, among others, are:

a. Making Media NA.

Making NA (Nutrient Agar) media is by dissolving 23 grams of NA powder into one liter of distilled water, the solution formed is put into a scott bottle then heated to homogeneous. Media NA was sterilized in autoclave for 15 minutes at 121 ^{0}C.

b. Making MCA Media.

Making MCA Media (Mac Conkey Agar) is to dissolve 23 grams of MCA powder, put it in a 50 mL beaker and dissolve it with distilled water and add it to Erlen meyer. Homogeneous by heating it in a heater for 15 minutes, then the Erlenmeyer mouth in the plug using cotton, coated with paper, then the blockage is closed again using paper, then tied. Sterilize the media in the autoclave at 121 ^{0}C for 15 minutes. Cool it by pouring it into a petri dish and storing it in the refrigerator.

c. Making SSA Media.

Making SSA Media (Salminella Shigella Agar) is by dissolving 23 grams of SSA powder, then insert it in 50 ml glass beaker and dissolve it with aquadest, then put it in Erlenmeyer. Homogeneous by heating it over pemabas for 15 minutes. Sterilize the media in the autoclave at 121 ^{0}C for 15 minutes. Chill and pour into a petri dish and store in the refrigerator.

d. Making MSA Media.

Making MSA Media (Manitol Salt Agar) is by dissolving 60 grams of dehydrate medium in 1000 ml of cold aquadest. Shake and pan until boiling (don't boil for too long) until it dissolves completely. No need to be sterile, pour it on a petri dish in a 50 ml glass beaker.

The making of media used in testing biochemical activities, among others, are:

a. Making TSIA media.

Making TSIA Media (Triple Sugar Iron Agar) Media, weighed TSI powder with analytical balance. TSI powder is put into erlenmeyer then dissolved with distilled water. The media solution is heated by using an electric stove and stirred until it dissolves

perfect. Check the pH using a pH stick, the pH of the TSI media is 7.4. The media is put into a test tube, 5 ml each using a measuring pipette. The tube is covered with fatty cotton, aluminum foil, and tied with pulung yarn. Media solution is sterilized by autoclave. The reaction tube is placed in a slanted position until the media freezes.

b. Making SIM media

SIM media is a medium consisting of sulfur and indol metility (tripton medium). So in making it, the first thing to do is:

1) Making motility: Prepare tools and materials for weighing 20 tons of Pepton, NH4 of citrate as much as 0.2 grams, N2S2O3 for 0.2 grams, agar for 0.3 grams, then add 1 liter of aquadest and warm up to boil.

2) Preparation of 1% tripton medium (Indol): weighing 10 grams of tripton, add 1 L of aquasest, then heat the boiling ground.

After both boils, mixing between Motility and Indol is done, after being mixed, it is cooled for a moment and put into a test tube. The media is ready for use with the puncture method.

c. Making the media Simon Citrat

Dissolve SC powder 22.5 g / L, autoclave 15 minutes at a temperature of 121 0C, then enter the saver and tilt it until it hardens, with a pH of 6.6 ± 0.2 at a temperature of 25 ^{0}C.

d. Making Urea media

Dissolve urea powder 21 g / L distilled water, autoclave (15 minutes 121 ^{0}C), cool to 45-55 ^{0}C and add 50 ml of 40% urea from 40% urea solution which is filtered and sterilized. Let it stand on its side until it hardens. PH 6.8 ± 0.2 at a temperature of 25 0C (Dwijoeseputro, 1990)

5. Isolation of bacteria

a. Wings of flies separated between the right and left wings, This applies to each type of fly that is sampled.

b. Each of the fly wing samples, put it in an eppendorf tube containing Phosphate Buffer Saline (PBS) or called a buffer solution. PBS is a solution consisting of weak acids and salts that can maintain and maintain pH. PBS solution consists of Sodium Chloride, Sodium Phosphate, Potassium Phosphate and Potassium Chloride dissolved with distilled water / water for injection (WFI). The phosphate group is useful to maintain pH.

c. The samples on the eppendorf tube were then cultivated into the prepared medium, namely the NA, MC and SS media for later identification of the growing microbes.

6. Microscopic Morphology Identification with Gram Staining

The object glass is cleaned with 96% alcohol then fixed on a spirit lamp, then the active isolate is taken aseptically and placed on the object glass and leveled. Re-fixation over a lamp of spirits. After chilling, Gram Gram (crystal violet) drops 2-3 drops for 1 minute, then washed with running water and dried in air. After that it is dripped with Gram B (Iodine) for 1 minute, washed with running water and dried in air. Then it is dripped with Gram C (96% alcohol) for 30 seconds, then washed with running water and dried in air. The last is dripped with Gram D 42 (Safranin) for 45 seconds, then washed with running water and excess water is removed with absorbent paper. This observation is done by looking at the shape and color of cells under a microscope with a certain magnification.

7. Testing Biochemical Activity

a. TSIA Test (triple sugar iron agar)

Microbial isolate culture was taken as much as one ose then scratch on the slant to see the nature of the microbes, incubated at 37 ° C for 24 hours.

b. Indol test

Microbial isolate culture was taken as much as one ose then inoculated into the medium to see the nature of the microbes, incubated at 37 ° C for 24 hours, then add kovak's reagent to see the color changes.

c. Motility Test

Microbial isolate culture was taken as much as one ounce then inoculated into the medium to see the nature of the microbes, incubated at 37 ° C for 24 hours.

d. Urea test

Microbial isolate culture was taken as much as one ose then scratch on the slant to see the nature of the microbes, incubated at 37 ° C for 24 hours.

e. Citrat Test

The microbial isolate culture was taken as much as one ose then scratch on the slant to see the nature of the microbe, incubated at 37 ° C for 24 hours (Dwijoeseputro, 1990) .

8. Molecular Identification

a. DNA extraction

DNA extraction is basically a series of DNA separation processes from other cell components. DNA extraction in eukaryotic organisms is carried out through sample preparation, lysis of cells (cell lysis), DNA binding, washing and elution.

The steps of DNA extraction are as follows:

1) A total of 200µl of sample was put into a micro centrifuge tube of 1.5 ml sterile and 20µl of Proteinase K. were added.

2) Homogeneous by pipetting, then incubated at 60 ^{0}C for 5 minutes.

3) Add 200µl of GSB Buffer (Geneaid) then vortex then incubated again at the same temperature for 2 minutes. 45 Then added absolute ethanol (96%) and vortex for 10 seconds.

4) Transfer all the mixture into a spin column, centrifugation at 14,000 xg for 1 minute. Remove the collection tube under the spin column and replace it with a new collection tube.

5) Add 400µl buffer W1 then centrifuge for 30 seconds at the same speed then remove the liquid that is in the collection tube.

6) Add 600µl of wash buffer (Geneid) centrifuge for 30 seconds, then remove the liquid in the collection tube and centrifuge it again for 3 minutes. Dispose of the collection tube and place a sterile microcentrifuge on the bottom of the spin column.

7) Next add 100 µl of Elution buffer to let sit for 3 minutes then centrifuge with the same speed for 30 seconds. DNA containing liquid stored in microcentrifuge tubes was stored at -4 ^{0}C to be used as a PCR template (Levinson, 2008) .

b. PCR Amplification (Polymerase Chain Reaction).

The process includes 3 stages, namely denaturation at 95 ^{0}C for 30 seconds, annealing at 55 ^{0}C for 30 seconds and extention at 72 ^{0}C for 1 minute. 46 This procedure was performed on isolated DNA samples, extracting DNA from the sample and aquadest as a negative control. "PCR mix" is inserted into the PCR tube:

Reaksi	**(µl)**
ddH2O	34.75
10X PCR buffer	5
25 mM MgCl	2 2
5 mM Dntp	1
Reverse primer (20pmol)	1
Forward primer (20pmol)	1
Hotstart DNA pol.	0.25
DNA sample	5
Total premix	50

Amplification is done using a PCR machine (DNA thermal Cycler). For PCR amplification, the initial stages of denaturation at 95 ºC for 15 minutes, then 94 ºC for 1 minute, annealing at 55 ºC for 30 seconds, extension of 72 ºC for 1 minute as many as 40 cycles followed by a final extension of 72 ºC for 5 minutes and 12ºC ± 30 minutes for storage.

c. Agarose gel electrophoresis

Agarosa is made by dissolving 2 g of agarose (BioRad) in 100 ml of 0.5 x Tris borate EDTA (100 g Tris base, 27.5 g boric acid, 20 ml 0.5 M EDTA pH 8.047 in 1 liter of water). Then it is heated to boiling and dissolves. Then added 1 µl of ethidium bromide (0.2 µg / ml) and put it in a comb-mounted gel printer. After agarose solidifies (about 30 minutes) then put it in an electrophoresis tank containing 0.5% TBE solution. Enter the sample DNA which has been mixed with a "loading dye" liquid into the well with a ratio of 2: 1, then put a 100 bp marker after the entire sample is inserted. The electrode is connected to the power supply and then turned on for 1 hour + voltage of 100 volts, 400 Ampere. After that, the electrophoresis device is turned off then the gel from the device is taken. The gel is transferred into the UV transiluminator then the results are observed on the computer. The size of the PCR amplification fragment was determined by comparing the position of the DNA marker size

(Marker) with the size of the sample fragment. Positive results are indicated by the presence of a band at 996 bp.

d. Sequencing Data Analysis

Sequencing data analysis is done using DNA star software program. For sequence alignment analysis, it is done by comparing the queries obtained with those already in the Gene Bank with the NCBI internet searches database (http // www.ncbi.nlm.nih.gov) using BLAST (Basic Local Alignment Search Tool).

STAGE II

Antagonistic test Pathogenic bacteria from fly wings with various kinds of bacteria obtained from other wings of each fly.

Antagonism testing in vitro

Antagonism test is carried out in vitro with a technique developed by Arwiyanto (2007). The antagonistic bacteria that are meant here are bacteria found in the wings of flies, while the bacteria found in other wings are bacteria that will be tested with the antagonist bacteria.

Antagonistic bacteria are grown by taking bacterial colonies using sterile toothpicks and then pointing to the YPGA medium which has been determined symmetrically from each other in a petri dish. The culture was incubated for 48 hours at 29oC. After incubating the cup is turned over and added 1 mL of chloroform, then left for 2 hours at room temperature. After all the chloroform vapor evaporates, the petri dish is reversed in its original position, then poured 200 µL of bacteria from the other wing in 4 mL of 0.6% water. The culture was incubated for 24 hours at 29oC to observe the inhibition zone. After the incubation period recorded isolates that produce inhibitory compounds are characterized by the presence of an inhibition zone around antagonistic bacterial colonies. The inhibition zone is measured and expressed in millimeters. Detection of the inhibitory mechanism is done by the method according to Arwiyanto (2007).

STAGE III

Anti-bacterial production of fly wings

Anti-bacterial Activity Test

Antibacterial activity test was carried out using agar diffusion method, a test bacteria derived from flies that had been grown pipette based on the number of cells into the NA medium, stirred with the stirrer and then poured in a petri dish and allowed to harden. Each extract in methanol is dropped every 10 µl on 6 mm disc paper. (The disc paper had previously contained bacteria derived from the wings of various types of flies obtained from STAGE II of this study), disc paper was left ± 15 minutes until methanol evaporated. After that, each paper disc is placed in a petri dish at a certain distance, according to the numbering at the base of the petri, to facilitate observation. The petri dish containing paper disc is then diffused in the refrigerator for ± 2 hours, after which it is put into an incubator for 2x24 hours at 37 ^{0}C (Abdel-Raouf & Ibraheem, 2008). Observations are made on the first day (after 1x24 hours) and the second day (after 2x24 hours). Isolates with the highest activity have the widest and not partial diameter of the clear zone.

VIII. PLAN OF DISCUSSION

1. Implementation of hadis in the Sahih Bukhary book no. 5336 in a systematic science framework, how to prove and process to make scientific frrame
2. The step of identification in the wings of the flies at the residential area of Gowa sulsel district ; morphological, biochemist, molecular.
3. The result of Antagonist test Pathogenic bacteria from fly wings against various kinds of bacteria obtained from other wings of each flies tudied ; how the process chemically and biologically.
4. anti-bacterial products from wings of flies ; explore of isolates which has highest activity and the widest clear zone

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