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by

Submission date: 19-Jun-2023 06:47AM (UTC+0700)
Submission ID: 2118512387
File name: A.1.b.1.1._Jurnal_IJSR_Vol_7_Issue_2_Peb_2018.pdf (231.05K)
Word count: 3283
Character count: 17715

International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2015): 6.391

Secondary Metabolite Compounds of Bee Pollen the Geniotrigona Incise Sakagami & Inoue. from Samarinda East Borneo

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Abstract: ***Secondary metabolites compounds of bee pollen a hive products Geniotrigona incise Sakagami& Inoue. has been investigated. Pollen samples extracted with ethanol by masseration method using a rotary evaporator. Ethanol obtained crude extract was fractionated by ethanol and ethyl acetate. Secondary metabolites of bee pollen with phytochemical analysis showed that the ethanol extract contains alkaloids and phenolic rough. The ethanol extract fraction containing alkaloids. Ethyl acetate extract fractions contain alkaloids and phenolic. In a test of an increase in mortality of larvae shrimp (Artemiasalina L.) and then processed using SAS Probit Analysis to determine the lethal concentration 50% (LC_{50}). The results were obtained data shows that the most active fractions is extract ethanol with value is 249.6079 ppm. Test results of antioxidant activity by DPPH method using spectrophotometry showed that 50% inhibitory concentration (IC_{50}) is obtained in the crude extract was 104.84 ppm Ethanol, ethanol fraction value is 172.67 ppm, 75.99 ppm of ethyl acetate fraction and vitamin C is 10.79 ppm, this suggests that the most active fraction is ethyl acetate with Ic50 is 75.99 ppm.***

Keywords: bee pollen, hive products, Geniotrigona incise Sakagami& Inoue., Secondary metabolites

1. Introduction

Health is an essential condition in the human body, therefore it is necessary to keep the intake that are beneficial to the body [1], [6]. One of the many useful meal intake, which is consumed by the public in order to preserve the health of her body is organic food that comes from a variety of honey bee hive products [6], [7], [9]. Honey bees, producing honey, Royal Jelly and Propolis, also produces pollen (Bee pollen) that has benefits for the health of the human body [7]. Bee pollen contains natural chemicals with complex compositions, bee pollen has the remarkable property of all kinds, including as an antioxidant [6], [7]. Optimum strength and durability of the body against various diseases may be obtained by adding 20% bee pollen on our food [1], [7], [13].

Bee pollen with the completeness of the elements of its nutrition value, working primarily on the cells metabolism [7], [13]. Based on previous research, in the analysis of ethanol extract of phytochemicals bee pollen flavonoid compounds contain positive and phenolic [8],[13]. But still not many who do research in depth about the chemical content of bee pollen, in particular chemical compounds analysis of secondary metabolites from bee pollen [8], [13] as well as to the utilization of bee pollen extracts as ingredients a safe alternative to drugs so the need was determined the level of toxicity. Based on the above description, then it has done the research to find out the types of compounds are secondary metabolites, a fraction of what most good value toksisitasnya with testing active against larval shrimps (Artemia salina L.), through the Brine Shrimp Lethality test Test (BSLT), to determine the effectiveness of each faction venom power [11] as well as to know the magnitude of the antioxidant activity of the radical method of soaking with 2.2-diphenyl-1-picrylhidrazyl (DPPH) extracts [7],[13] from pollen (bee pollen) produced by Geniotrigona incise Sakagami & Inoue.

2. Materials and Methods

The materials used are beepollen of Geniotrigona incise Sakagami & Inoue, ethanol, ethyl acetate, hexane, chloroform, diethyl ether, 2 M H_2SO_4, glacial acetic acid, Bi (NO_3) 3.5 H_2O, $HgCl_2$, concentrated HNO_3, KI, $FeCl_3$, HCl, Aquades Mg, powder, sea water, DMSO, DPPH (2.2-diphenyl-1-picrylhidrazyl) and Vitamin c.

The equipment used in this research is a rotary evaporator, a beaker, erlenmeyer flask, glass measuring cup, funnel, separating funnel, analytical balance, test tubes, pipette, pipette volume drops, micropipette 100-1000 μL size, measure, stir pumpkin, paper Whatman # 1 filter, aluminum foil, lamp TL, hot plate, freezer and UV-Vis spectrophotometer.

3. Procedure

Samples pollen (Bee pollen) from the bees Geniotrigona incisa Sakagamie & Inoue which had dried upweighed, then macerated with ethanol, extracted to extract solution colorless again. Then filtered and the solvent was evaporated with the Rotary evaporator thus obtained a rough extracts ethanol. Next extract the ethanol rough fractionated use solvents with a moderately difference solvent. The trick is as follows:

- Extract the rough non ethanol ethanol has added a mixture of ethanol and ethyl acetate in comparison with 1:2 (v/v).
- Performed with oil bath funnel, so retrieved 2 fraction, i.e. The fraction of ethanol and ethyl acetate fraction.

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- Concentrated with ethyl acetate fraction of the rotary evaporator and are known as ethyl acetate fraction extract.
- Ethanol concentrated fractions with the rotary evaporator and is referred to as extract fraction ethanol.
- On a rough and second extract fraction (fraction of ethanol and ethyl acetate fractions) then conducted trials of phytochemicals and shrimp larvae mortality trials (brine shrimp lethality test).
- Next extract crude and second fractions is performed using the antioxidant activity assay method of soaking radicals 2.2-diphenyl-1-picrylhidrazyl (dpph) by using a spectrophotometer.
- In this study as a positive control, to test the power of antioxidants in comparison of extracts of bee pollen extract against rude, the fraction of ethanol and ethyl acetate fraction, use the default value of antioxidant vitamin c content values of ic_{50} vitamin c is an antioxidant level is the best among the other extracts.

4. Results and Discussion

The sample used in this study was the pollen grains (bee pollen) from the bees Geniotrigona incisa Sakagamie & Inoue which had dried up. The taking of the test material is granular bee pollen was done manually by selecting a beehive box lot has a bowl containing bee pollen, bee pollen BLOB-shaped pure solids, which is wrapped by materials resin (propolis).

To simplify the process of retrieving blobs of bee pollen from the wrapper, i.e. in a way put the Bowl contains bee pollen on the space cooling so that the wrap propolis hardens, making it easier the process of separation. Samples that have been collected and then dried in a container of sterile and done in a closed room to avoid direct sunlight for approximately 24 hours. After that the sample is crushed and mashed, feathered sample aims to maximize the interaction of ethanol (solvent) and samples of bee pollen so expect the overall secondary metabolites can be extracted, after sampling mashed then the samples that have been finely weighted [8], [14].

After the sample extract beepollen macerated using ethanol, this process is done in a dark colored bottle tightly closed, so that the ethanol that is used as the solvent does not evaporate into the air. The process of maceration is carried out for approximately 5 minutes. The process of maceration is causing solvent ethanol will penetrate the cell wall so that it can go into the cavity-cell cavity containing a wide variety of compounds are secondary metabolites. The solution of the maceration was filtered then evaporated (concentrated) the solvent with the rotary evaporator, so that the obtained extract, extract fraction of ethanol and ethyl acetate fraction extract of bee pollen, which was yellow-brown.

4.1 Test of Phytochemicals

To find out the content of secondary metabolite compounds in samples of pollen (bee pollen) then it needs to be done testing of extracts and phytochemicals from the second fraction extract oil results i.e. fraction of ethanol and ethyl acetate fraction extract of bee pollen. Test of phytochemicals which are done among other things test the alkaloids, flavonoids, phenolic substances, saponins, steroid and triterpenoid.

The test results on the test material in the form of phytochemicals extract crude, the fraction of ethanol and ethyl acetate fraction of bee pollen is qualitatively indicates the presence of secondary metabolite compounds. Test results showed that phytochemicals in coarse extract contained compounds, alkaloids and phenolic. In the fraction of ethyl acetate contained phenolic alkaloids and compounds, while the fraction of ethanol only secondary metabolite compounds alkaloids only.

4.2 The BSLT Test (Brine Shrimp Lethality Test)

To observe the power of bee pollen extract toxic need to do testing of shrimp larvae mortality (Brine Shrimp Lethality Test). Shrimp larvae Mortality test is a method of testing by using test animals i.e. meat, shrimp Artemiasalina (l.) can be used as power determination of toxicity of a compound. According to the observations of potential bioactivityhas been done based on the Lethal Concentration 50% (LC_{50}) is a value which indicates the concentration of toxic substances that could result in the death of the organism up to 50%.

When the LC_{50} 30 ppm then extract < was extremely toxic and potentially contains anticancer bioactive. Meyer (1982) mentions the level of toxicity of an extract:

- $LC_{50} \leq 30$ ppm = Very toxic
- 31 ppm $LC_{50} \leq 1{,}000$ ppm = Toxic
- $LC_{50} > 1{,}000$ ppm = Not toxic

Based on the test results of shrimp larvae mortality extract coarse bee pollen obtained the value of LC_{50} = 355.46 ppm; on extract fraction of ethanol obtained the value of LC_{50} = 249.60 ppm and fraction of ethyl acetate extracts on the retrieved value of LC_{50} = 276.35 ppm. This value indicates that the concentrations, extracts from bee pollen is able to kill the larvae of shrimp up to 50% of the population. LC_{50} value of shrimp larvae mortality test obtained using Probit Analysis SAS.

Based on data from test results that indicate that mortality of bee pollen extract fraction of ethanol has highest bioactivity against the larvae of shrimp that is indicated by the value of the smallest i.e. LC_{50} 249.60 ppm. This value indicates that at concentrations 249.60 ppm, bee pollen extract fraction ethanol capable of killing shrimp larvae up to 50% of the population. The smaller the value of LC_{50} (Lethal Concentration 50%) of a sample of the high toxicity.

The high activity of the toxicity of ethanol extracts of bee pollen fractions against shrimp larvae compared to extract crude and bee pollen extract fraction of ethyl acetate, is estimated to be due to the alkaloid compounds are quite high, it is due to the alkaloid compounds ethanol fraction on a more active phase in polar [1], [10], [12]. On a rough

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value of bee pollen extract LC_{50} acquired for 355.46 ppm which means bioactivity on rough bee pollen extracts was lower compared with the fraction of ethanol and ethyl acetate fraction.

Although the toxicity of extract and bee pollen extract fraction of ethyl acetate ethanol fraction less than toxicity. But based on the study conducted Meyer (1982) [6], the chemical compound is said to be potentially active when it has a value of LC_{50} less than 1,000 ppm. Thus it can be said that extract crude, the fraction of ethanol and ethyl acetate fraction of potentially active because the value of the resulting LC_{50} less than 1,000 ppm.

4.3 Antioxidant test with the method of DPPH

To know the magnitude of the DPPH radical curbs percentage (% inhibition) extracts from pollen (bee pollen) then conducted trials with antioxidant activity method of DPPH radical curbs [14]. According to [6], [8] in the observation of potential antioxidant bioactivity was done based on the value of the Inhibitations Concentration 50% (IC_{50}) was a value which indicates the concentration of antioxidant activity (% of AA) obtained from measurements absorbance and then retrieved a radical DPPH arrest percentage of 50%. DPPH radical insoluble in ethanol and has a strong absorbance at a wavelength of 517nm with purple. After reacting with the DPPH antioxidant compounds, will be reduced and the color will change to yellow. The results of the change from purple to yellow was proportional to the number of electrons that are captured. Following the reaction of DPPH and antioxidants:

After having obtained the optimum wavelength on 517nm, then performed in order to obtain the value of absorbance measurement percentage of DPPH radical curbs by using the following formula for measuring percent curbs radical DPPH (% of AA):

$$\%AA = 100 - \{[(AB - AA)] \times 100 / AKN\}$$

The results of the analysis and results of calculations note that on rough bee pollen extract has antioxidant potential with the category are bee pollen extract and fraction of ethanol has the potential antioxidants with weak category, This indicated value of IC_{50} (concentration inhibition) obtained at the rough 101-150 of ethanol extract of ppm and fraction obtained from ethanol are more in the range of 151ppm, i.e. with IC_{50} values of acquisition 104.84 ppm and bee pollen extracts on the fraction of ethanol 172.67 ppm. The results of the test of bee pollen extract fraction of ethyl acetate IC_{50} value obtained is amounting to 75.99 ppm, so the antioxidant activity of the extract owned bee pollen ethyl acetate fraction categorized strong, due to the value of the IC_{50} values were owned at the range of 50-100 ppm.

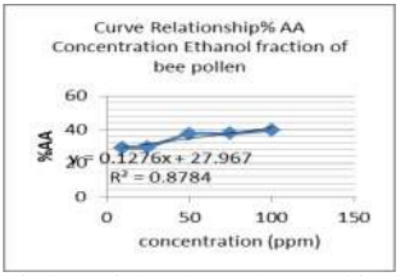

Figure 1: Graph of IC_{50} values on the ethanol fraction of bee pollen

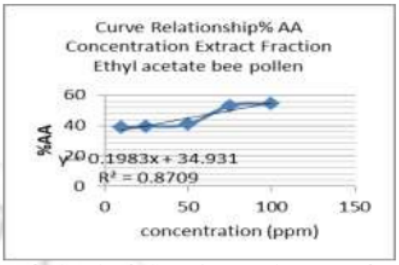

Figure 2: Graph of IC_{50} values on the extract fraction ethyl acetate of bee pollen

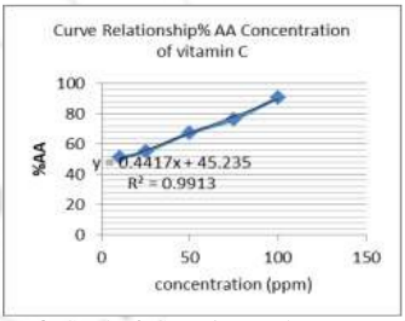

Figure 3: Graph of IC_{50} Values on the concentration vitamin C of bee pollen

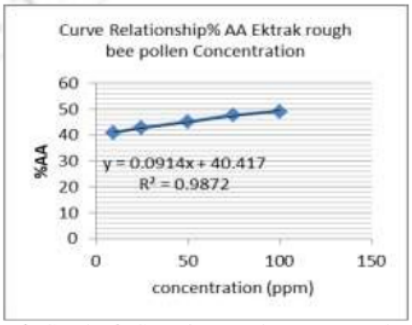

Figure 4: Graph of IC_{50} values on the extract crude of bee pollen

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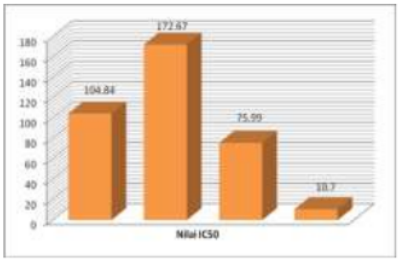

Figure 5: Graph of IC_{50} Values on the Graph image extract crude, the fraction of ethanol and ethyl acetate fraction, and vitamin C

On the chart above, it can be seen that the values of IC_{50} of extracts of rough and the two factions namely the fraction of ethanol and ethyl acetate fraction are all greater than vitamin C, this fact gives an interesting fact that bee pollen extract was not a the pure compound, but still contains other compounds that may not have antioxidant activity. The lower the IC_{50} value means the higher the antioxidant activity. Specifically, a compound described as very strong antioxidants in IC_{50} values of less than 50 ppm, strong in the values of IC_{50} between 50-100 ppm, is when the value of the IC_{50} between 101-150 ppm and weak if the value of IC_{50}lebih from 151 ppm.

If the test result against bee pollen extracts antioxidant activity was associated with the value of LC_{50} obtained, it can be known that bee pollen extract fraction of ethyl acetate has the most powerful antioxidant activity with a value of 75.99 ppm and the LC_{50} value bee pollen extract obtained from ethyl acetate fraction was 276.35 ppm. From the results obtained show that at concentrations of 75.99 ppm, bee pollen extract fraction of ethyl acetate is able to capture radical DPPH amounting to 50%. While LC_{50} obtained show that at concentrations 276.35 bee pollen extract ppm ethyl acetate fraction was capable of killing shrimp larvae up to 50% of the population. Although the bee pollen extract on ethyl acetate fraction have greater antioxidant activity compared to extract crude and ethanol fraction bee pollen extract, but the fraction of ethyl acetate was not secure because the value as antioxidants under the LC_{50} of 1000 ppm or categorized are toxic.

5. Conclusion

Secondary metabolite compounds bee pollen extracts, namely, alkaloids and phenolic extracts, both of ethanol or ethyl acetate fraction and bee pollen extracts on ethanol fraction only alkaloid compound. Based on the values obtained from the results of toxicity testing of bee pollen extract obtained against the value of LC_{50} best bee pollen extracts on ethanol fraction with a value of 249.6079 ppm and obtained the results from trials of antioxidant extracts of IC_{50} values obtained bee pollen with ethyl acetate fraction the highest value (75.99 ppm).

Acknowledgments

To the Ministry of education and culture, through the Research center of Mulawarman University over financing higher education research grant TA-2014, on Research Grants compete with Skim Decree; No, 211/UN17-16/PG/2015.

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