Stability Test of Colouring Agent from Pericarpium of Red Dragon Fruit (*Hylocereus Polyrhizus*) Extract in Laboratory Diagnostic of Intestinal Nematode Eggs Preparation

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Abstract. The red dragon fruit pericarpium contains anthocyanin as a natural red dye agent that can be extracted from plant cells. Anthocyanin can be used as a colouring agent in intestinal nematode worm eggs. The aim of the research is to determine a stability test of natural red dye produced from 3\% red dragon fruit pericarpium extract after storing for 27, 28, 29 weeks using a spectrophotometer at 504 nm wavelength and also to determine a stability test in diagnostic application in colouring of intestinal nematode worm eggs preparation. The result of the research indicated the longer the storage time, the lower the absorbance value obtained. Likewise with preparations, the longer the storage of these dyes the less the quality of the dye. The processing data used Anova test. From the result of the statistical test can be concluded that stability of the dye agent will be decrease according to length of time required. The suggestion for next result is to do resistance test for a long time accurately.

1. Introduction
The red dragon fruit (*Hylocereus Polyrhizus*) is very good for health consuming. This fruit has a pericarpium (skin) that has not widely used so that it can cause environmental pollution. As an alternative to reduce pollution, dragon fruit pericarpium waste can be used as a natural coloring agent in laboratory diagnostic for coloring intestinal nematode eggs [1-3,6,8,10,12-14,16,19].

Dragon fruit pericarpium contains anthocyanin as a natural coloring agent. A pericarpium of dragon fruit can be isolated by extraction to get anthocyanin agent. And then the result of extraction, anthocyanin agent, can be determined quantitatively by using spectrophotometer at 504 nm wavelength. [1-4,6-14]

In the previous research, we did extraction process, quantitative determination of anthocyanin and coloring of intestinal nematode eggs. In coloring worm eggs preparation step, we used coloring agent anthocyanin with 1\%, 2\%, 3\%, concentration respectively and did the assessment score system namely score 1 and score 2. In this research, we done again like previous research for coloring in worm eggs preparation. The concentration of anthocyanin used was 3\% because the concentration of 1\% dan 2\% had been tried but unfortunately worm eggs were not colored well. The purpose of this research was to do again the coloring in worm eggs preparation using anthocyanin and then to do stability test for 27, 28, 29 weeks for determination the quality of worm eggs preparation. [2,4,6-14,16,18,19]

The diagnostic of intestinal nematode worm eggs from feces can be done by using Natif method [8]. In the Natif method we used eosin as a coloring agent for intestinal nematode worm eggs. Eosin...
has a function as an agent for coloring worm eggs because worm eggs containing protein with alkaline condition so that can be chemically bound with eosin in acid condition. It is the same way with anthocyanin extraction from dragon fruit pericarpium because anthocyanin has a same characteristic with eosin. [1-2,5-9,12-15,17,19]

2. Methods
Research conducted march 2019, located at Medical Analyist School of Bakti Asih, Bandung Laboratory. Research method was experimental. Tools used in the study were bulb pipette, glass object, microscope, cover glass, spectrophotometer, tissue, spraying bottle, volumetric flask, and erlenmeyer. Material used were aquadest, dragon fruit of pericarpium extraction 3%, eosin, and feces.

Firstly, we made the dilution of 10 ppm dragon fruit of pericarpium extraction from 3% dragon fruit of pericarpium extraction. To make the dilution of 10 ppm we pipetted 0.3 ml extract and then adding by aquadest until 250 ml in the volumetric flask. We continued making dilution of 1 ppm dragon fruit of pericarpium extraction by pipetting 2.5 ml the dilution of 10 ppm extract and then adding by aquadest until 25 ml in the volumetric flask. Next we continued making the dilution 2 ppm of dragon fruit of pericarpium extraction by pipetting 5 ml the dilution of 10 ppm extract and then adding aquadest until 25 ml in volumetric flask. The next step, we made the dilution of 3 ppm, 4 ppm, 5 ppm dragon fruit of pericarpium extraction from the dilution of 10 ppm.

Secondly, we made determination of maximum wavelength by putting the results of dilution of dragon fruit pericarpium extract 1 ppm to 5 ppm into the spectrophotometer and then measured with a spectrophotometer with a wavelength of 400-800nm. We continued measuring absorbance by putting results of dilution of the extract of dragon fruit pericarpium 1 ppm to 5 ppm into the spectrophotometer and then read at the wavelength that has been obtained.

Thirdly, we made manufacture of Eosin controls for coloring worm eggs, by weighing 2 grams of Eosin and then adding by 100 ml aquadest.

Fourthly, we made coloring nematode intestinal worm eggs (Natif method) using eosin. by preparing samples and materials and then picking up the tip of a stool (feces) sample stick and placing it on a glass object. Dropping 1 drop of alternative coloring (red dragon fruit pericarpium extract) homogeneous. Then, covering with cover glass. Then, observing under a 400x magnification microscope.

Fifthly, we made coloring of intestinal nematode worm eggs (Natif method) using 3% alternative coloring anthocyanin 1%. Preparing samples and materials then pinched the end of a stick of feces samples and placing it on a glass object and then dropping 1 drop of alternative coloring (Red Dragon Fruit pericarpium Extract) homogeneous. Then, covering with cover glass. Then, observing under a 400x magnification microscope.

3. Result and Discussion
The following result of research:

27th Weeks

| Control using eosin 2% | Concentration of anthocyanin 3% |
|-----------------------|--------------------------------|

Figure 1. Worm eggs of *Ascaris Lumbricoides*  
Figure 2. Worm eggs of *Ascaris Lumbricoides*
**Table 1. Absorbanse value**

| No | Concentration | Repeating | 27th | 28th | 29th |
|----|---------------|-----------|------|------|------|
| 1  | 1ppm          | 1         | 0.0060 | 0.0049 | 0.0026 |
|    |               | 2         | 0.0057 | 0.0038 | 0.0023 |
| 2  | 2ppm          | 1         | 0.0092 | 0.0076 | 0.0061 |
|    |               | 2         | 0.0088 | 0.0074 | 0.0057 |
| 3  | 3ppm          | 1         | 0.0120 | 0.0113 | 0.0087 |
|    |               | 2         | 0.0118 | 0.0099 | 0.0079 |
| 4  | 4ppm          | 1         | 0.0207 | 0.0196 | 0.0187 |
|    |               | 2         | 0.0204 | 0.0192 | 0.0177 |
| 5  | 5ppm          | 1         | 0.0266 | 0.0253 | 0.0238 |
|    |               | 2         | 0.0257 | 0.0246 | 0.0229 |
Estimated Marginal Means of Absorbance

![Estimated Marginal Means of Absorbance](image)

**Figure 7.** Graphic of Absorbance

### Table 2. Observing preparat

| Control (Eosin 2%) | 27th weeks | 28th weeks | 29th weeks |
|-------------------|------------|------------|------------|
| 2                 | 1          | 1          | 1          |
| 2                 | 1          | 1          | 1          |
| 2                 | 1          | 1          | 1          |
| 2                 | 1          | 1          | 1          |
| 2                 | 1          | 1          | 1          |
| 2                 | 2          | 2          | 2          |
| 2                 | 2          | 2          | 2          |
| 2                 | 2          | 2          | 2          |
| 2                 | 2          | 2          | 2          |
| 2                 | 2          | 2          | 2          |
| 2                 | 2          | 2          | 2          |
| 2                 | 2          | 2          | 2          |
| 2                 | 2          | 2          | 2          |

In this study, stability testing of dragon fruit extract after 27, 28, 29 weeks was carried out at
temperature 20 Celsius absorbance was measured using a spectrophotometer to determine the quality of worm egg preparations which were colored by anthocyanin 3% dyes before taking absorbance measurements, dragon fruit peel extract is diluted first. It aims to reduce the concentration of the dragon fruit extract from 3% to 10 ppm. After that measurements used at various concentrations 1, 2, 3, 4, 5 ppm diluted from 10 ppm. Absorbance value can be showed in table 1. From table 1 and figure 7 it can be stated that the higher the concentration of dragon fruit pericarpium extract the higher the absorbance value and the longer the storage time carried out also had an impact on the decrease in absorbance value, so the longer it is stored the more the fading color of the dragon fruit pericarpium extract.

After obtaining the absorbance value, staining of intestinal nematode worm eggs using Natif method. Then viewed under a microscope with a magnification of 400x. To facilitate reading in the field of view, using a rating method with score 1 and score 2. Score 1 stated unclear color and morphological form, background and egg color could not be distinguished. Score 2 stated clear color and morphological form, background and egg color could be distinguished.

The following assessment of staining of worm egg preparations under a microscope after 27, 28, 29 weeks for a score of 1 can be caused by suspension of dirty eggs or damage worm eggs so that no contrast visibility and also the cover glass was not tightly closed because there was so the color was not homogenous. At score 2, eggs absorb dyes because the substances that has the same characteristic with eosin acid. The stability of the dyes after 27.28 and 29 weeks is still quite good but has decreased from the initial quality. The factors that influence the degradation of the quality of dyes are sunlight radiation, lamp light, temperature etc. [1-6]

4. Conclusion
Based on experiments that had been done, it can be concluded that in worm egg preparations there are differences in the quality of the eosin dye 3% after storing 27th, 28th, 29th week at temperature 20 celcius. Absorbance values can affect the quality of anthocyanin dyes because according to anova test results with sig value 0.000 < 0.05 that can be accepted.

5. Sugestion
After conducting research on the stability test of dyes for red dragon fruit peel extract we should to continue the reseach to determine resistance test for anthocyanin as a natural coloring agent.

References
[1] Alfrida 2018 Karakteristik Zat Warna Antosianin dari Biji Kakao Non Fermentasi Sebagai Sumber Zat Warna Alam. Dalam Jurnal [online] 8 Halaman, Tersedia: https://media.neliti.com/media/publications/171938-ID-pengaruh-waktu-maserasi-zat-antosianin-s.pdf [25 Desember 2018]
[2] Asep 2005 Ekstraksi Filtrasi Membran Dan Uji Stabilitas Zat Warna dari Kulit Manggis. Dalam Jurnal [Online] 8 Halaman, Tersedia: https://www.academia.edu/6281852/Ekstraksi_Filtrasi_Membran_dan_Uji_Stabilitas_Zat_Warna_dari_Kulit_Manggis_Garcinia_Mangostana_Asep_Muhamad_Samsudin_L2C005239_dan_Khoiruddin_L2C005271 [25 Desember 2018]
[3] Cahyadi, w.2009 Analisis dan Aspek Kesehatan: Bahan Tambahan Pangan, Bumi Aksara: Jakarta
[4] Greenwood, & Norman N., & Earnshaw, &Alan., 1997. “Chemistry of the Elements, 2nd Edition, Oxford, Butterworth, Heineman”.
[5] Hambali, E. & Mujdalipah, S & Tambunan, A. H & Pattiwiridan, A. W & Hendroko, R., 2008. “Teknologi Bionergi” Jakarta : Agro Media.
[6] Hambali, M. & Mayangsari, F. & Noermansyah, F., 2014 “Ekstraksi Antosianin dari Ubi Jalar Dengan Variasi Solven, Dan Lama Waktu Ekstraksi”. Jurnal Teknik Kimia 20, (2), 25-35.
[7] Harborne, J.B., 1987, MetodaFitokimia: Penentuancara Modern Mengalisis Tumbuhan, Terbitan Kedua, ab. K Padmawinata dan I. Soediro. Penerbit ITB, Bandung
[8] Hasanah, H. 2013. *Artikel Ilmiah Gambaran Telur Cacing Nematoda Usus Beserta Siklus Hidup* (online) Tersedia : PDF Jurnal STH – Agustus 2017 Gambaran Telur Cacing Nematoda Usus.

[9] Hendayana, Sumar.1994.*Kimia Analitik Instrumen.Semarang:*IKIP Semarang Press.

[10] Jackman, R.L. and J.L. Smith. 1996. *Anthocyanins and Betalains.* di Dalam Natural Food Colorants. Hendry, G.A.F. dan J.D. Houghton (ed.). Blackie Academic &Profesional, London

[11] Khopkar, S. M. 1983. *Konsep Dasar Kimia Analitik (Terjemahan).* Bombay : Indian Institute of Technology.

[12] Kristanto. 2003.*Buah Naga, Pembudidayaan Di Pot Dan Di Kebun. Swadaya. Cimanggis.* Depok.

[13] Lydian 2014*Stabilitas Antosianin Jantung Pisang Kepok(Musa paradisiaca L) Terhadap Cahaya Sebagai Pewarna Agar-Agar.* Dalam jurnal [online] 8 halaman. Tersedia: https://jurnal.ugm.ac.id/agritech/article/download/9431/7005 [25 desember 2018 ]

[14] Mahmudatussa’adah 2014*Karakteristik Warna dan Aktivitas Antioksidan Antosianin Ubi Jalar ungu.* Dalam jurnal IPB[online] 9 Halaman, Tersedia : journal.ipb.ac.id/index.php/jtip/article/download/9109/7164 [ 3 Januari 2019]

[15] Manjang, Y. 2004. *Penelitian Kimia Organik Bahan Alam, Pelestarian dan Perkembangan Melalui Tanah Agrowisata,* Workshop Peningkatan Sumber Daya Manusia Penelitian dan Pengelolaan Sumber Daya Hutan yang Berkelanjutan. Pelaksana Kelompok Kimia Organik Bahan Alam Jurusan Kimia FMIPA Universitas Andalas Padang Kerjasama Dengan Proyek Peningkatan Sumber Daya Manusia ditjen diktidepknas

[16] Nida 2013 *Kandungan Antosianin Dan Aktivitas Antioksidan Ubi Jalar Ungu Segar Dan Produk Olahannya.* Dalam Jurnal UGM[Online] 7 Halaman, Tersedia : https://jurnal.ugm.ac.id/agritech/article/download/9551/7126 [ 25 desember 2018]

[17] Sudjadi. 1988. *Metode Pemisahan.* Yogyakarta: Kanisius.

[18] Underwood, A. L. dan R.A. Day Jr. 1989. *Analisis Kimia Kuantitatif* (Diterjemahkan oleh R. Soendoro). Jakarta.

[19] Yohana 2016 *Ekstraksi Antosianin dari Kulit Buah Manggis(Garcinia mangostana L)Sebagai Zat Warna Alami.* Dalam Jurnal [online] 27 Halaman. Tersedia : http://digilib.uin-suka.ac.id/22341/1/09630026_bab-i_iv-atau-v_Daftar-Pustaka.pdf [ 23 Oktober 2018 ]

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