COMPARISON OF SPECTROPHOTOMETRIC AND
TLC-DENSITOMETRIC TECHNIQUE IN DETERMINATION
OF PHYTOMELATONIN IN GREEN ALGAE (Spryrogyra sp)
ETHANOLIC EXTRACT

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Abstract

Background. Instrumental analysis method gave a difference measurement results in
determination of content level of active substance in medicinal herb.

Objective. The aim of this study is to compare the level content of phytomelanin in green
algae extract determined by spectrophotometry and TLC-densitometry technique.

Methods. The phytomelanin was extracted from green algae using 96% aethanol. The
qualitative Alkaloid screening were done by using Mayer and Dragendorff test. The quantitative
determination of phytomelanin were done spectrophotometrically at 277 nm wavelength and using
TLC densitometry technique with silica gel GF 254 plate and eluated by BAW (n-bathanol: acetic
acid: water=12:3:5 v/v). The spots were scanned at 254 nm wavelength.

Outcome measured. Phytomelanin level in aethanolic extract of green algae

Results. The results showed that the content phytomelanin content of green algae level by
spectrophotometric technique was 0.22±0.01 % and 0.88±0.04 % was assayed by TLC-densitometric.

Conclusion. The TLC-densitometry technique gave the higher phytomelanin of green algae
level than spectrophotometry technique (p<0.05)

Keywords: Phytomelanin, green algae, aethanolic extract, spectrophotometry,
TLC-densitometry
INTRODUCTION

Green algae contains melatonin called as phytomelanotin, a substance that wide used for cancer prevention, antioxidant (Veronique et al., 2005), surpassing the myocardial damage due to nicotine (Baykan et al., 2008), anti-mouthcancer (Varvares, 2008), prevention of bleeding in the brain (Koh, 2008), inhibited the neurotoxic than arsenic (Lin et al., 2008), prevent kidney damage due to smoking (Ozan et al., 2007) and antihypertensive (Xia et al., 2008).

Based on its chemical structure, the phytomelanotonin can dissolve in ethanol. Phytomelanotonin can be determined by spectrophotometry and TLC-densitometry technique.

This study aims to compare the phytomelanotonin level in ethanolic extract of green algae determined by spectrophotometry and TLC-densitometry technique.

MATERIAL AND METHOD

Material

The main material was green algae (Spyrogyra sp). Chemical material: absolute ethanol p.a., petroleum ether, ether, acetic acid p.a., HCl, n-butanol p.a., purchased from Merck, Dragendorf dan Meyer reagents, aquaest dan Silica gel GF-254 plate.

Methods

1. Plant identification

Plant identification was done at Laboratorium Ilmu Alam Fakultas MIPA Universitas Ahmad Dahlan.

2. Sample collecting

Green algae were collected from Rowo Jombor, District Bayat, Klaten regency, Central Java in March of 2012.

3. Aethanolic Extract preparation

Phytomelanotonin was extracted from green algae by Soxhlet apparatus with ethanol then evaporated with rotary evaporator to obtain thick extract. The water content and the ash content of the aethanolic extract were determined by gravimetric technique.

4. The aethanolic extract Purification

About 15 mL 15% acetic acid was added into the thick aethanolic extract, then filtered using Buchner funnel. Wash the filtrate with petroleum ether. The acetic acid layer were separated and added NH4OH until the pH value was 10. Pour 50 mL of ether into the basic solution. Remove the water layer. Evaporoporate the ether layer to obtain the residue. Dissolve the residue using aethanol. The absorbance of the solution were measured at 277nm wavelength.

5. The screening of alkaloid and identification of phytomelanotonin in aethanolic extract

The alkaloid screening were done using Dragendorf and Mayer test. The formation of sediment after the addition of the dragendorf or mayer reagent into the acidic sample indicated the presence of alkaloid in the sample.

The qualitative analysis of phytomelanotonin in the aethanolic extract was done with TLC technique. The similarity Rf value between the phytomelanotonin standart spot and sample spot indicated the presence of phytomelanotonin in the sample.

6. The quantitative analysis of phytomelanotonin

a. Spectrophotometry technique

The phytomelanotonin standart solutions in many various level were prepared. The interval level were between 0.1-0.3mg/mL. The absorbance of various level of phytomelanotonin standart solution and the purified aethanolic extract were measured at 277nm wavelength.
using Pharmaspec UV 1700 (SHIMADZU) spectrophotometer.

The linear regression equation between the level of standart phytomelatonin vs absorbance was determined. This equation was used to calculated the level of phytomelatonin in aethanolic extract.

b. TLC-densitometry thecuine

The silica F254 was used as stationary phase. The phytomelatonin standart (0.2-2.0/mg/ml) and the aethanolic extract were eluted with BAW (12.3:5)w/v. The AUC (area under the curve ) values were determined by scanning the dried spot with TLC scanner 3 (CAMAG) at 284nm wavelength. The linear regression equation between the level of phytomelatonin standart vs AUC was determined. This equation was used to calculated the level of phytomelatonin in the aethanolic extract.

RESULT AND DISCUSSION

The plant identification result showed that the plant used int his study was green algae (Spirogyra sp.).

About 24,3399 grams aethanolic extract (rendemen 9.74%) was produced from 250 grams green algae. The water content of the extract was 8.34% dan 3.06% of ash content.

The alkaloid screening, both of Dragendorf and Mayer tests indicated that the aethanolic extract of green algae consist of phytomelatonin.

The qualitative analysis of phytomelatonin in aethanolic extract by TLC thecuine showed the presence of phytomelatonin in both of the purified aethanolic extract and aethanolic extract. (fig.1)

The quantiative analysis of phytomelatonin

a. Spectrophotometry technique

The spectrophotometry technique was based on the ability of the phytomelatonin content in the aethanolic solution to absorb the electromagnetic radiation in Ultra Violet region. The maximum wavelength of the phytomelatonin standart was 277nm. The spectra of purified aethanolic extract showed the maximum wavelength at 275.8nm and the spectra profile showed the similarity between phytomelatonin standart and the purification aethanolic extract spectra. It's showed that the purified aethanolic extract consist of phytomelatonin.

The absorbance of phytomelatonin standart solution in many various level was available in table I. The graphic fig. 2 showed the correlation between the phytomelatonin standart level Vs the absorbance.(p<0.05).
Table I. The absorbance of the phytomelatonin standard solution

<table>
<thead>
<tr>
<th>C (mg/10ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>0.286</td>
</tr>
<tr>
<td>0.15</td>
<td>0.412</td>
</tr>
<tr>
<td>0.20</td>
<td>0.543</td>
</tr>
<tr>
<td>0.25</td>
<td>0.688</td>
</tr>
<tr>
<td>0.30</td>
<td>0.715</td>
</tr>
</tbody>
</table>

The qualitative parameter of the chromatogram were the Rf value. The Rf value of the phytomelatonin standard was 0.75 and the Rf value of the aethanolic extract was 0.76. It's indicated that the aethanolic extract consist of phytomelatonin. The quantitative parameter of the TLC-densitometry technique were The AUC values. The AUC values of the phytomelatonin standard were described in table III.

Figure 2. The graphic correlation between phytomelatonin level vs the absorbance

![Graph showing correlation](image)

Figure 3. The chromatogram of phytomelatonin standard (1-6) and aethanolic extract of green algae (a-c) on silica F254 plate after eluated by BAW (12:3:5)w/v under UV detector

Table II. The phytomelatonin level in aethanolic extract of green algae determinated by spectrophotometry technique

<table>
<thead>
<tr>
<th>Sample weight (mg)</th>
<th>Abs</th>
<th>Phytomelatonin level (%)</th>
<th>$\bar{x}$ (%)</th>
<th>SD</th>
<th>CV (%)</th>
<th>$\bar{x} \pm Le$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>998.6</td>
<td>0.469</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>998.8</td>
<td>0.482</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>998.8</td>
<td>0.489</td>
<td>0.23</td>
<td>.22</td>
<td>$5.48 \times 10^{-3}$</td>
<td>2.74</td>
<td>$0.22 \pm 0.01$</td>
</tr>
<tr>
<td>995.1</td>
<td>0.494</td>
<td>0.23</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>998.8</td>
<td>0.475</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

b. The TLC-densitometry technique

The chromatogram result from the TLC technique was described at figure 3.
Table III. The AUC values of the phytomelatonin standard

<table>
<thead>
<tr>
<th>No</th>
<th>Phytomelatonin level (mg/ml)</th>
<th>AUC (mV)</th>
<th>Linear regression</th>
<th>R value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>9.1528</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.6</td>
<td>19.5458</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>27.2703</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.4</td>
<td>29.7615</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1.8</td>
<td>32.9887</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.0</td>
<td>32.3886</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Y = 12.48 x + 10.63  
R = 0.94

Figure 4. The graphic correlation between the phytomelatonin level Vs AUC.

TLC-densitometry technique more sensitive than spectrophotometry technique. Another advantage of TLC-densitometry technique was the selectivity. The TLC-densitometry technique was more selective than spectrophotometry.

CONCLUSION

The TLC-densitometry technique gave the higher phytomelatonin of green algae level than spectrophotometry technique (p<0.05)

Table IV. The phytomelatonin level in aethanolic extract of green algae determined by spectrophotometry technique

<table>
<thead>
<tr>
<th>Sample weight (mg)</th>
<th>AUC (mV)</th>
<th>Phytomelatonin level (%)</th>
<th></th>
<th>SD</th>
<th>CV (%)</th>
<th></th>
<th>± Le (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>997.8</td>
<td>31.8568</td>
<td>0.85</td>
<td>0.88</td>
<td>0.04</td>
<td>4.5</td>
<td>0.88 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>995.5</td>
<td>31.6834</td>
<td>0.85</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1000.2</td>
<td>32.6445</td>
<td>0.88</td>
<td>0.88</td>
<td>0.04</td>
<td>4.5</td>
<td>0.88 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>999.1</td>
<td>32.1614</td>
<td>0.86</td>
<td>0.86</td>
<td>0.04</td>
<td>4.5</td>
<td>0.88 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>895.9</td>
<td>31.8769</td>
<td>0.95</td>
<td>0.88</td>
<td>0.04</td>
<td>4.5</td>
<td>0.88 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

According to The data in the table I showed that the TLC-densitometry technique gave the higher value of the phytomelatonin level in the aethanolic extract of green algae.(p<0.05) It indicated that the

ACKNOWLEDGEMENT

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