PROCEEDING

THE INTERNATIONAL CONFERENCE ON
SAFETY MANAGEMENT OF CENTRAL CYTOTOXIC RECONSTITUTION IN PHARMACY PRACTICE

MAY 25TH, 2013
HOTEL GRAND TJOKRO YOGYAKARTA

MAY 26TH-27TH, 2013
BETHESDA HOSPITAL YOGYAKARTA

Published by :
FACULTY OF PHARMACY
AHMAD DAHLAN UNIVERSITY YOGYAKARTA
INDONESIA

COMPARISON OF SPECTHROPHOTOMETRIC AND TLC-DENSITOMETRIC TECHNIQUE IN DETERMINATION OF PHYTOMELATONIN IN GREEN ALGAE (*Sprirogyra* sp) ETHANOLIC EXTRACT

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Abstract

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

Objective. *The aim of this study is to campare the level content of phytomelatonin in green algae extract determinated by spectrophotometry and TLC-densitometry technique.*

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

Outcome measured. *Phytomelatonin level in aethanolic extract of green algae*

Results. *The results showed that the content phytomelonin 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 phytomelatonin of green algae level than specthrophotometry technique ($p<0.05$)*

Keywords : *Phytomelatonin, green algae, aethanolic extract, spectrophotometry, TLC-densitometry*

ISBN : 978-979-18458-6-1

INTRODUCTION

Green algae contains melatonin called as phytomelatonin, 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 it's chemical structure, the phytomelatonin can dissolve in ethanol. Phytomelatonin can be determinated by spectrophotometry and TLC-densitometry technique.

This study aims to campare the phytomelatonin level in ethanolic extract of green algae determinated by spectrophotometry and TLC-densitometry thechnique

MATERIAL AND METHOD

Material

The main material was geen algae (*Spyrogyra sp*). Chemical material : absolute ethanol p.a., petroleum ether, ether, acetic acid p.a, HCl , n-butanol p.a.. purcashed from Merck, Dragendrof dan Meyer reagents, aquadest 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 algaes were collected from Rowo Jombor , District Bayat, Klaten regency, Central Java in March of 2012.

3. Aethanolic Exctract preparation

Phytomelatonin was extracted from green algae by Soxhlet apparatus with aethanol then evapotated with rotary evaporator to obtaine thick extract. The water content and the ash content of the aethanolic extract were determinated by gravimetric thecnique.

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. Evapoporate 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 phytomelatonin 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 phytomelatonin in the aethanolic extract was done with TLC thecnique. The similarity Rf value beetween the phytomelatonin standart spot and sample spot indicated the presence of phytomelatonin in the sample.

6. The quantitative analysis of phytomelatonin

a. Spectrophotometry thecnique

The phytomelatonin standart solutions in many various level were prepared. The interval level were between 0.1-0.3mg/ml. The absorbance of various level of phytomelatonin standart solution and the purified aethanolic extract were measured at 277nm wavelength

ISBN : 978-979-18458-6-

using Pharmaspec UV 1700 (SHIMADZU) spechtophotometer

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

b. TLC-densitometry thecnique

The silica F254 was used as stationary phase. The phytomelatonin standart (0.2-2.0/mg/ml) and the aethanolic extract were eluated with BAW (12:3:5)v/v. The AUC (area under the curve) values were determinated by scanning the dried spot with TLC scanner 3 (CAMAG) at 284nm wavelength. The linear regreesion equation between the level of phytomelatonin standart vs AUC was determinated. This equation was used to calculated the level of phytomeltonin 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 aetaholic extract by TLC thecnique showed the presence of phytomelatonin in both of the purified aethanolic extract and aethanolic extract. (fig.1)

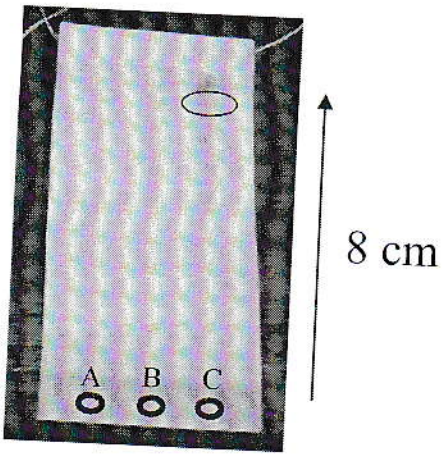

Figure 1. Chromatogram of phytomelatonin strandart (A), purified aethanolic extract (B) and aethanolic extract(C)

The quntitative analysis of phytomelatonin

a. Spectrrophotometry 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 mximum wavelength at 275.8nm and the spectra profile showed the similarity between phytomelatonin standart and the purification aethanolic extrct spectra. It's showed that the purified aethanolic extract consit of phytomelatonin.

The absorbance of phytomelatonin standart soution in many various level was available in table I. The graphic fig. 2 showed the corelation between the phytomelatonin standart level Vs the absorbance.($p<0.05$).

ISBN : 978-979-18458-6-1

Table I. The absorbanceof the phytomelatonin standart solution

C (mg/10ml)	Absorbance
0.10	0.286
0.15	0.412
0.20	0.543
0.25	0.688
0.30	0.715

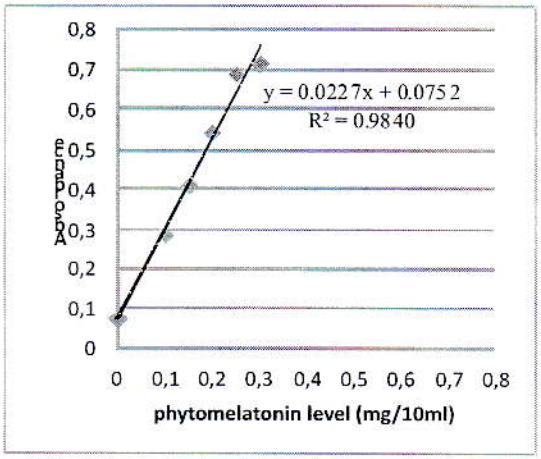

Figure 2. The graphic corelation between phytomelatonin level vs the absorbance

The qualitative parameter of the chromatogram were the Rf value. The Rf value of the phytomelatonin standart 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 phytomeltonin standart were described in table III.

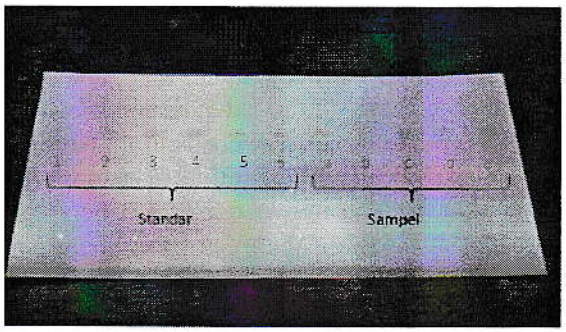

Figure 3. The chromatogram of phytomelatonnin standart (1-6) and aethanolic extract of green algae (a-e) on silica F254 plate after eluated by BAW (12:3:5)v/v under UV detector

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

Sample weight (mg)	Abs	Phytomelatonin level (%)	$\bar{x}$ (%)	SD	CV (%)	$\bar{x} \pm Le$ (%)
998.6	0.469	0.22				
998.8	0.482	0.22				
998.8	0.489	0.23	.22	5.48×10^{-3}	2.74	0.22 ± 0.01
999.1	*0.494*	*0.23*				
998.8	0.475	0.22				

b. The TLC-densitometry technique

The chromatogram result from the TLC technique was desribed at figure3.

ISBN : 978-979-[illegible]

Table. III. The AUC values of the phytomelatonin standart

No	Phytomelatonin level (mg/ml)	AUC (mV)	Linear regression	R value
1	0.2	9.1528		
2	0.6	19.5458		
3	1.0	27.2703		
4	1.4	29.7615	Y = 12.48 x + 10.63	0.94*
5	1.8	32.9887		
6	2.0	32.3886		

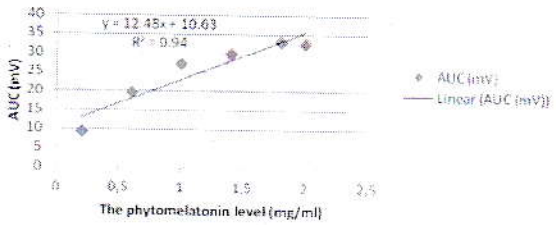

Figure 4. The graphic corelation between the phytomelatonin level Vs AUC

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 specthrophotometry technique ($p<0.05$)

Tabel IV. The phytomelatonin level in aethanolic extract of green algae determinated by spectrophotometry technique

Sample weight (mg)	AUC (mV)	Phytomelatonin level (%)	$\bar{x}$ (%)	SD	CV (%)	$\bar{x} \pm Le$(%)
997.8	31.8568	0.85				
995.5	31.6834	0.85				
1000.2	32.6445	0.88	0.88	0.04	4.5	0.88 ± 0.04
999.1	32.1614	0.86				
895.9	31.8769	095				

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

We would like to thank DP2M dikti and LPP UAD with funding this research

ISBN : 978-979-18458-6-1

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