INTERNATIONAL CONFERENCE ON CENTRAL MANAGEMENT OF CENTRAL CYTOTOXIC RECONSTITUTION

By HARI SUSANTI

PROCEEDING

INTERNATIONAL CONFERENCE ON CENTRAL MANAGEMENT OF CENTRAL CYTOTOXIC RECONSTITUTION

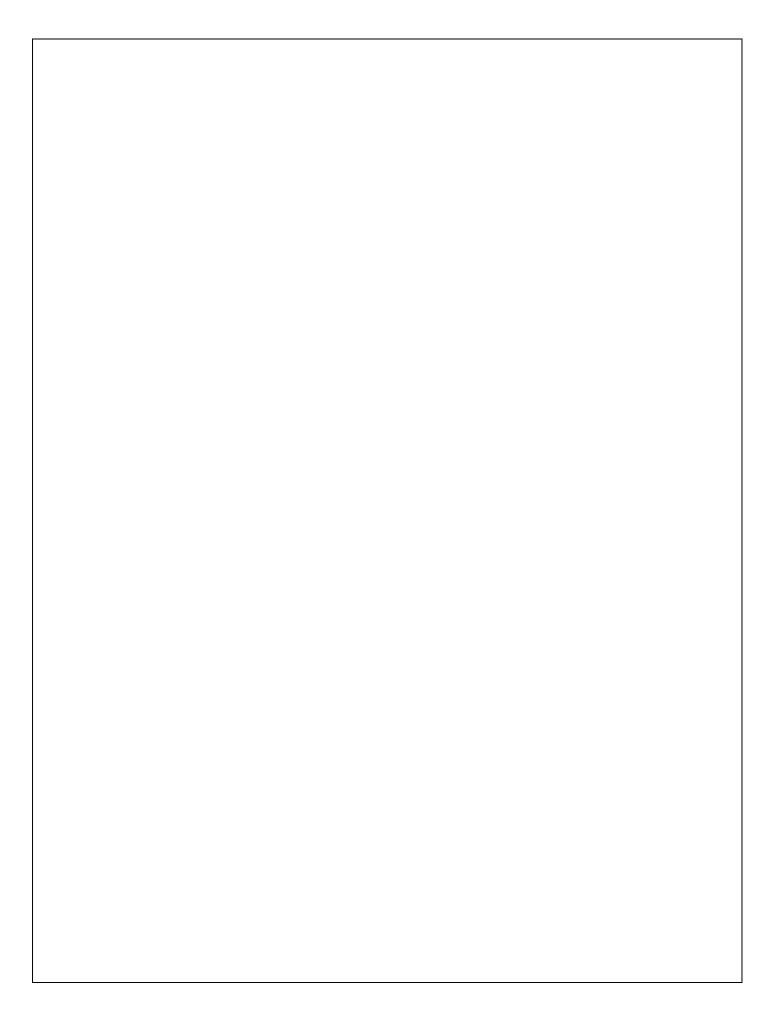
Grand Cokro Hotel Yogyakarta May 25th, 2013

THE INTERNATIONAL CONFERENCE ON CENTRAL MANAGEMENT OF CENTRAL CYTOTOXIC RECONSTITUTION IN PHARMACY PRACTICE YOGYAKARTA, INDONESIA, 2013

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Welcome Address from Chairman of Organizing Committee

Hono 4 ble Rector of Ahmad Dahlan University,
Dean Faculty of Pharmacy of Ahmad Dahlan University,
Honorable Plenary Speakers
Dear Colleague,
6 stinguished Participants,
Ladies and Gentlemen

Assalamu'alaikum warahmatullahi wabarakatuh,

Good morning,

First of all, let us give thanks to Allah, the Almighty God, who has allowed us to attend this conference. Secondly, I would like to welcome everyone to Yogyakarta.

Preparation and reconstitution of drug products are an essential function of hospital pharmacy. The hospital pharmacist should also take notice for central intravenous additive service including cytotoxic reconstitution services. Thus, handling cytostatic in hospital is also crusial in hospital practises. In other words, Cytotoxic Handling should to provide protection to patients, the operator and environment.

Ladies and gentlemen,

To celebrate the 17th aniversary faculty of Pharmacy Ahmad Dahlan University, Yogyakarta, in collaboarting with Bethesda Hospital, Yogyakarta, Indonesia hosted The International Conference on Safety Management of Central Cytotoxic Reconstitution in Pharmacy Practice". The conference is held on 25 May, 2013 at The Grand Tjokro Hotel, Yogyakarta, Indonesia. The conference facilitated some of professional that come from world wide such as academia, researchers, hospital pharmacists, policy maker, and health care professionals. The second agenda is Workshop on Basic Cytotoxic Handling for hospital pharmacists and academia. It will hold on 26-27 May, 2013 in Pharmacy Department, Bethesda Hospital.

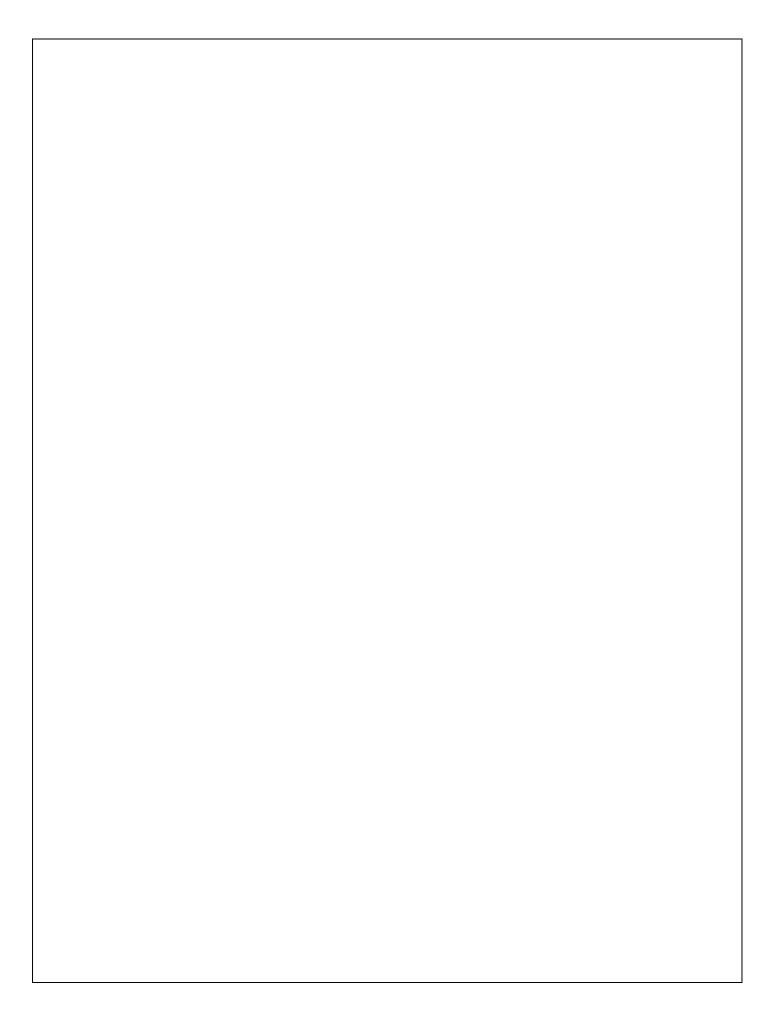
I do hope this conference will give a new initiative for practicing sorounding cytotoxic handling and team-work. I thank to conference sponsors and committee members for their support.

Wassalamu'alaikum warahmatullahi wabarakatuh,

Yogyakarta, 25 May 2013

Dr.rer.nat. Endang Darmawan, M.Sc., Apotheker

Chairperson of the organizing committee



Welcome Address from Dean of Faculty of Pharmacy, Ahmad Dahlan University

Assalamu'alaikum Wr. Wb.

Dear all participants,

Welcome to the International Conference and Workshop on Safety Management of Central Cytotoxic Reconstitution in Pharmacy Practice. Thank you for participating in this great event. Hopefully, you can enjoy the days of International Conference and Workshop.

The topic of this international seminar is very interesting and important for the development of pharmacists' skills in drug reconstitution, since we know that our new perspective of pharmacy practice is patients' care and patients' safety. Dealing with cytotoxic drug is a kind of collaboration among physician, pharmacist, nurse, psychologist, patient and patient's family. However, as the pharmacists, we have unique skills and knowledge related to the preparation, administration and monitoring of cytotoxic drug administration. Therefore, in a team work, pharmacist has some responsibilities as a leader related to the cytotoxic drug reconstitution.

This international conference and workshop are the collaboration between Faculty of Pharmacy, University of Ahmad Dahlan Yogyakarta as academic institution with the Bethesda Hospital of Yogyakarta as one of the central cytotoxic reconstitution. We also invited Prof A.A. Kaptein, from Leiden University Medical Center, Leiden, The Netherlands and Harbans Kaur Dhillon from University Malaya, Medical Centre, Malaysia as speakers, besides Dra. L. Endang Budiarti, M.Pharm., Apt. as practitioners in Bethesda Hospital of Yogyakarta and Dr. Dyah Aryani Perwitasari, Apt., PhD who wants to share about development of pharmacist's skill in medical reconciliation. After the one day seminar, we invite you to joint with us on the workshop of Central Cytotoxic Reconstitution which will be held in Bethesda Hospital of Yogyakarta. This workshop is useful for us, especially when we want to start the Central Cytotoxic Reconstitution in our hospital.

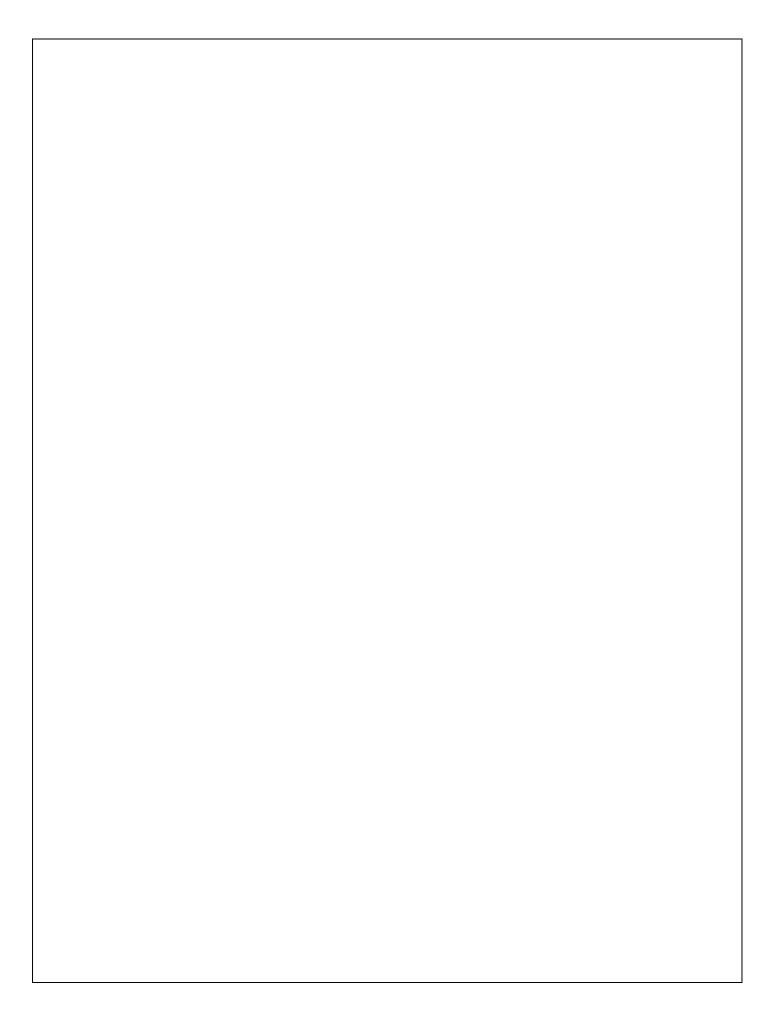
I hope, The International Conference and Workshop on Safety management of Central Cytotoxic Reconstitution in Pharmacy Practice will inspire us to practice our skills and knowledge in our fields. In the future, we can reach one the goal of pharmacy practice, which is patients' safety.

Have a great conference.

Wassalamu'alaikum wr wb

Dean of Faculty of Pharmacy University of Ahmad Dahlan, Yogyakarta

Dr. Dyah A Perwitasari, Apt., Ph.D

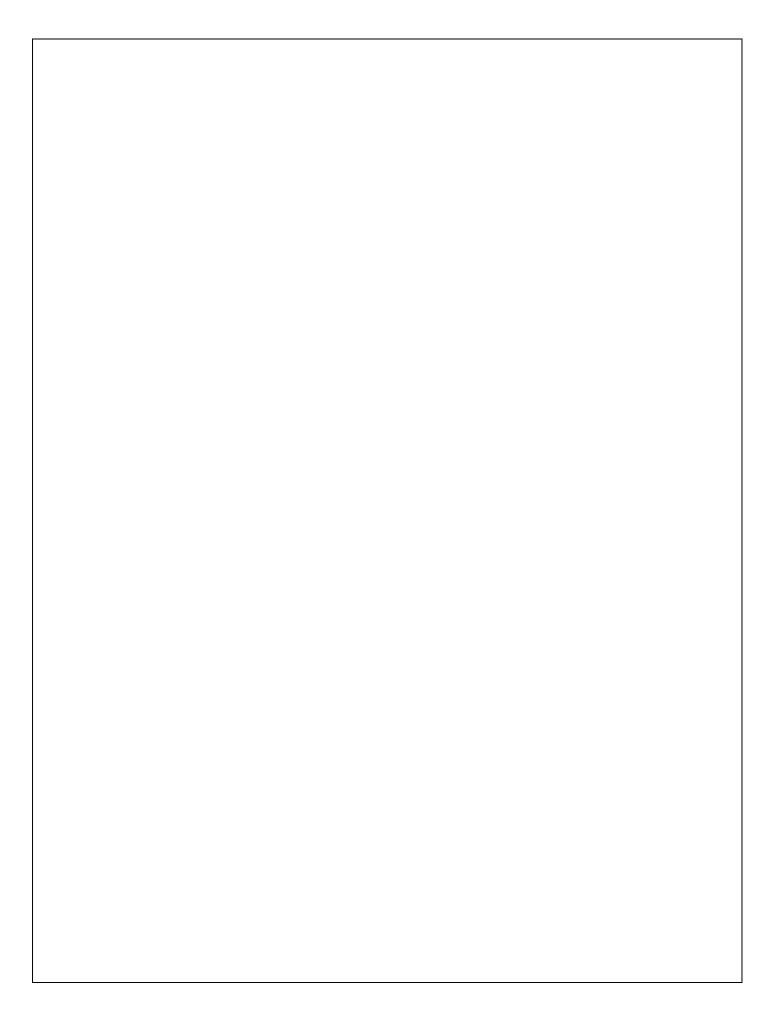


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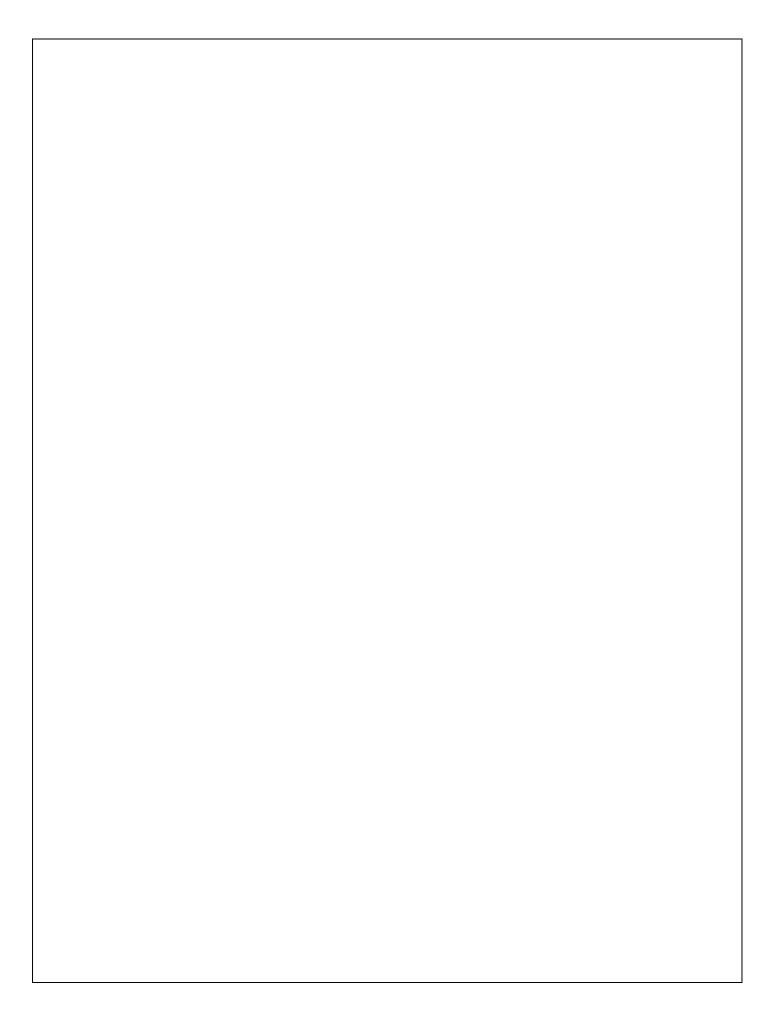
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COMPARISON OF SPECTHROPHOTOMETRIC AND TLC-DENSITOMETRIC TECHNIQUE IN DETERMINATION OF PHYTOMELATONIN IN GREEN ALGAE (Sprirogyra sp) ETHANOLIC EXTRACT

Hari Susanti, Wahyu Widyaningsih, Nina Salamah, Beta Zudia Fertaveni, Efi Puspitasari

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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

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 theorique.

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

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 phytomelatonin phase. The 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 AUC standart VS 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)

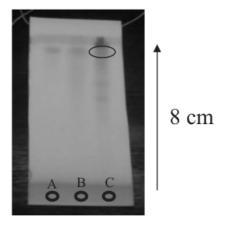


Figure 1. Chromatogram of phytomelatonin strandart (A), purified aethanolic extract (B) and aethanolic extract(C)

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. maximum wavelength of phytomelatonin standart was 277nm. The spectra of purified aethanolic extract showed the mximum wavelength at 275.8nm and the spectra showed the similarity between phytomelatonin standart and the purification aethanolic extrct spectra. It's showed that the purified aethanolic extract consit 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).

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

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 phytomelatonin standart were described in table III.

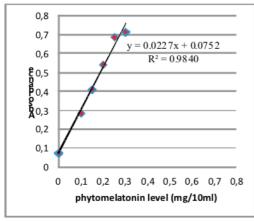


Figure 2. The graphic corelation between phytomelatonin level vs the absorbance

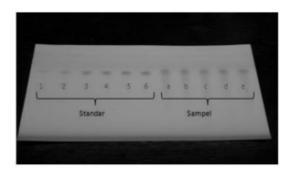


Figure 3. The chromatogram of phytomelatonnin standart (1-6) and aethanolic extract of green algae (a-e) on silica F254 plate after eluated by BAW (12:3:5)v/v under UV detector

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

Sample weight (mg)	Abs	Phytomelato nin level (%)		SD	CV (%)	x ± Le (%)
998.6	0.469	0.22				
998.8	0.482	0.22				
998.8	0.489	0.23	.22	5.48 x10 ⁻³	2.74	$0.22 \pm\ 0.01$
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.

Phytomelatonin level AUC No R value Linear regression (mg/ml) (mV) 0.2 1 9.1528 2 0.6 19.5458 3 1.0 27.2703 Y = 12.48 x + 10.630.94* 4 1.4 29.7615 5 1.8 32.9887 32.3886 6 2.0

Table. III. The AUC values of the phytomelatonin standart

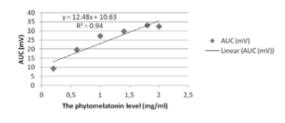


Figure 4. The graphic corelation between the phytomelatonin level Vs AUC

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)	Phytomelato nin level (%)	- x (%)	SD	CV (%)	x ± Le (%)
997.8	31.8568	0.85				
995.5	31.6834	0.85	0.88			
1000.2	32.6445	0.88			4.5	0.88
999.1	32.1614	0.86		0.04		± 0.04
895.9	31.8769	095				3.01

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

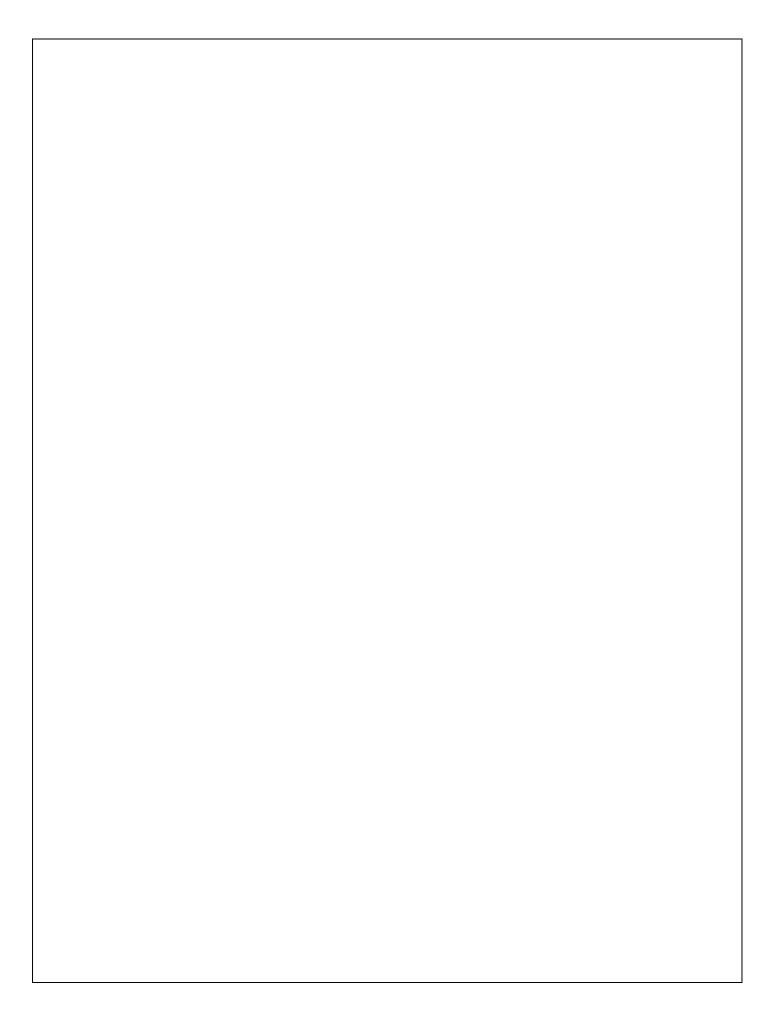
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