

# INDIFFERENCE ANTIBACTERIAL EFFECT OF BETEL (PIPER BETLE L.) EXTRACT IN COMBINATION WITH CIPROFLOXACIN AGAINST PSEUDOMONAS AERUGINOSA

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## ABSTRACT

**Purpose.** This research aimed to know the antibacterial effect of betel (Piper betle L.) in combination with ciprofloxacin against Pseudomonas aureus. P. aeruginosa resistance to some antibiotics, multidrug resistant P. aeruginosa (MDRPA) is a problem in treating bacterial infection. Ciprofloxacin is the most active antibiotic against P. aeruginosa, but it becomes resistance. A new compound or combination of compounds are required to overcome MDRPA. The combination of antibacterial compounds from plant with antibacterial drug were expected to increase antibacterial activity resulted in the reduction of resistance. Betel has antibacterial properties, this study measured the antibacterial effect of betle in combination with ciprofloxacin.

**Methods.** The antibacterial effect of betel extract and ciprofloxacin separately and the combination of both materials were evaluated based on the zone of inhibition diameter using agar diffusion method. The interaction effect of betel extract and ciprofloxacin combination was performed using microdilution checkerboard method.

**Results.** There was an antibacterial effect of betel extract started at the concentration of 150 mg/ml. Treatment with a combination of drugs has been used as an approach to overcome bacterial resistance, but the combination of betel extract with ciprofloxacin showed lower inhibition growth of P. aeruginosa compared than betel or ciprofloxacin alone. The addition of betel extract to ciprofloxacin didn't reduced the Minimum Inhibitory Concentration (MIC) of ciprofloxacin. The value of Fractional Inhibitory Concentration Index (FICI) is 3, there combination of betel extract and ciprofloxacin showed indifference interaction.

**Conclusion.** There was an indifferent antibacterial effect of the betel extract in combination with ciprofloxacin against P. aeruginosa. It isn't recommended to combine ciprofloxacin with betel on the infectious treatment of P. aeruginosa resistance to ciprofloxacin.

**Key words:** Piper betle, L., ciprofloxacin, indifferent antibacterial effect, Pseudomonas aeruginosa

## INTRODUCTION

The current problem of bacterial resistance to antibiotics suggests that a single compound has not been able to overcome bacterial infections. A new antibacterial or combination of compounds may resolve bacterial resistance problem. The combination of compounds can have synergistic, additive, or antagonistic effects. Stronger antibacterial effects can be achieved by combining the drugs[1-2] but the effect of betel and red betel combination showed antagonistic antibacterial activity against Staphylococcus aureus, Staphylococcus epidermidis, or Eschericia coli.[3] In the area of bacterial resistant, many researchers have been developing the natural extract as materials especially from plant extract which were combined with antibiotics.[4]

Pseudomonas aeruginosa is one of the bacteria that causes nosocomial infections. P. aeruginosa resistant drug (MDRPA) is a condition in which a bacterium resistance to three or more classes of antibiotics.[5] It was reported that P. aeruginosa resistance to several antibiotics, such as Ceftazidime (25.32%), Cefotaxime (37.97%), Ceftriaxone (37.97%), Ciprofloxacin (27.85), Gentamicin (26.58%), and others.[6] Ciprofloxacin is an antibiotic that belongs to the second most active class of fluoroquinolones against P. aeruginosa [7]

There have been no previous studies on antibacterial effect of the combination of betel (Piper betle, L.) extract with ciprofloxacin. In this study, we aimed to determine the antibacterial effect of betel extract and ciprofloxacin combination against Pseudomonas aeruginosa.

## MATERIAL AND METHOD

The research material was betel leaf taken from Sleman Yogyakarta Indonesia, and P. aeruginosa (bacteria concentration was equal to Mac Farland II standard), Mueller Hinton. Determination of red betel plant was done in Faculty of Biology Universitas Gadjah Mada, Yogyakarta, Indonesia. Herbarium of Piper betle L. is deposited at Pharmacy Laboratory, Faculty of Pharmacy, Sanata Dharma University, Yogyakarta, Indonesia.

Extraction with maceration followed by two times remaseration using methanol solvent. Evaporation was done with a rotary evaporator to obtain a thick betel extract. Testing of antibacterial activity was performed by agar diffusion method. Determination on the antibacterial effect type of the test material combination was performed by microdilution checkerboard method to establish a single ciprofloxacin MIC (MICA), MIC of a single betel extract (MICB), MIC ciprofloxacin in combination (MICAB), MIC extract of betel in combination (MICBA). Fractional Inhibitory Concentration Index (FICI) values were obtained by the formula: (MICA/MICAB) + (MICB/MICBA). Combination of ciprofloxacin and extract is called synergy effect if the value of FICI ≤ 0.5; whereas the term of indifference and antagonism are indicated if FICI > 0.5, and FICI > 4 respectively.

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## RESULTS

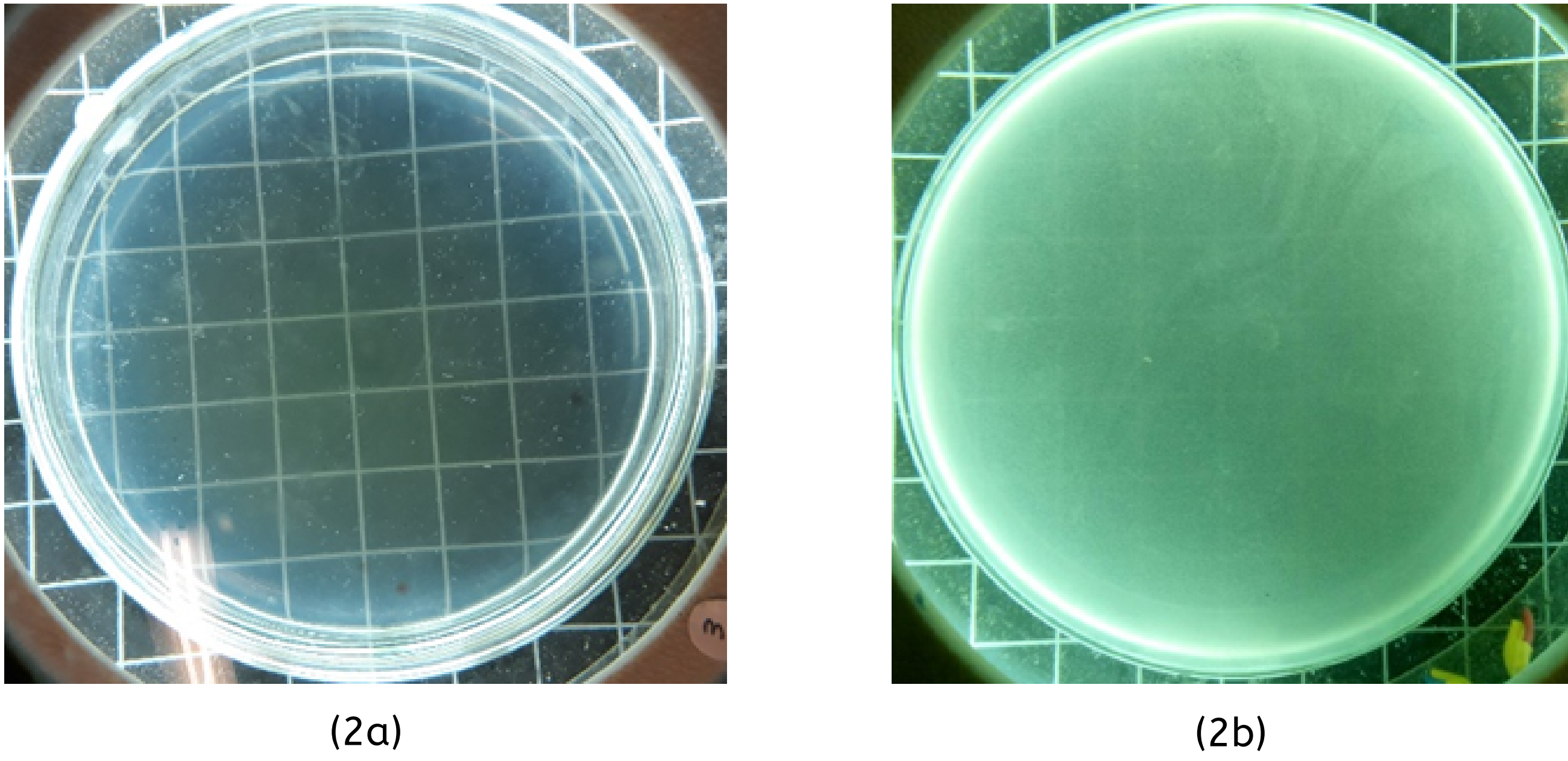


Figure 2. Control of medium sterility (a) and control of P. aeruginosa growth in the medium (b)

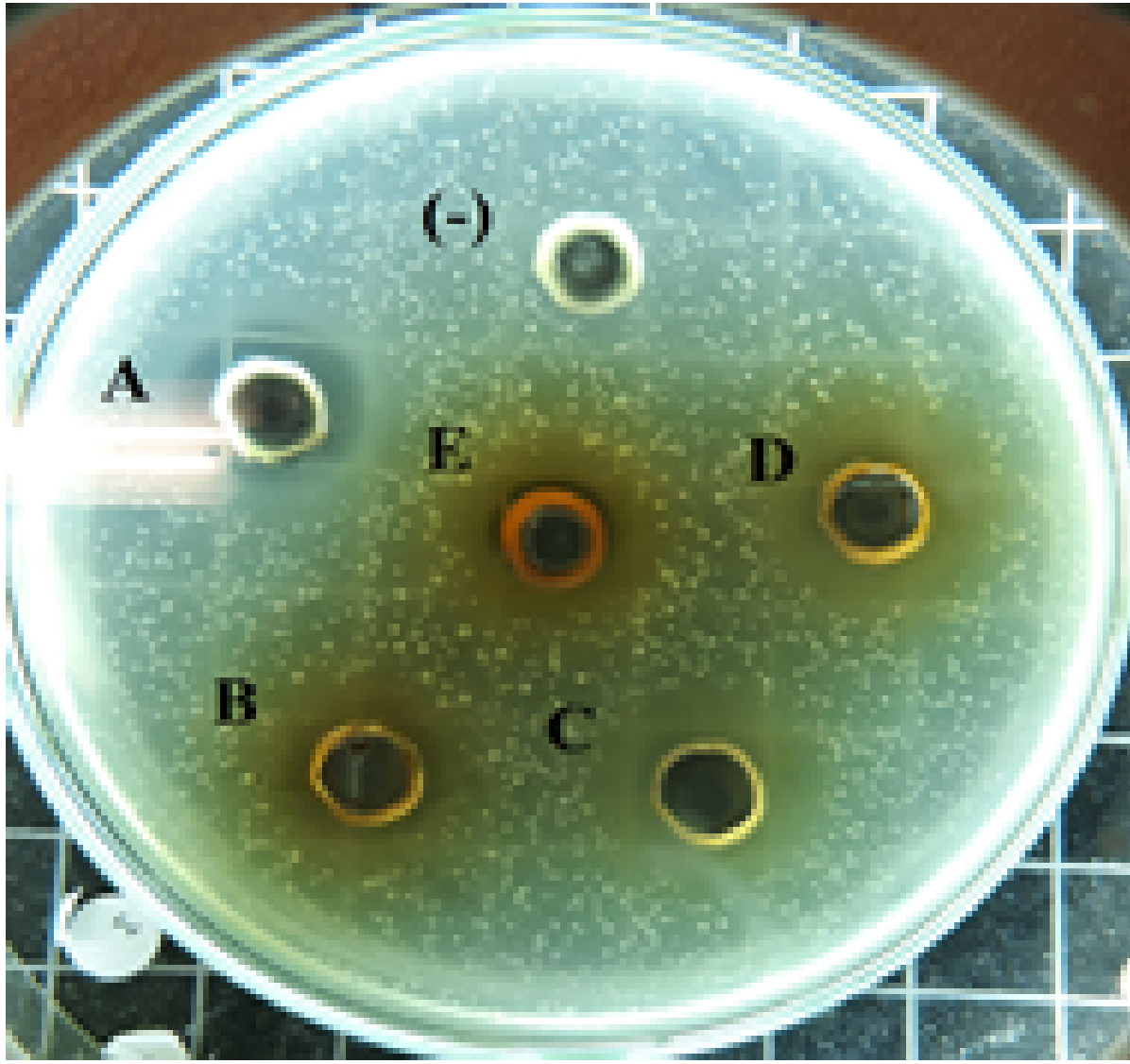


Figure 3. Inhibition zones of extract, antibiotic, and combination of extract and antibiotic against P. aeruginosa (A. Ciprofloxacin 0.5 µg/mL; B. betel extract (150 mg/mL), C. Combination extract and ciprofloxacin (150 mg/mL : 0.5 µg/mL), D. Combination extract and ciprofloxacin (300 mg/mL : 0.5 µg/mL), E. Combination extract and ciprofloxacin (600 mg/mL : 0.5 µg/mL), (-) DMSO 1%, aquadest steril, and BPW

**Tabel I.** results of antibacterial effect of betel extract and ciprofloxacin combination against P. aeruginosa with microdilution checkerboard method

		Betel extract (mg/mL)			
Concentration		0	150	300	600
Ciprofloxacin (µg/mL)	0	0,572*	0,104	0,742	0,491**
	0.5	0,021**	0,451	0,858	0,580
	1	0,017	0,450	0,763	0,543**
	2	0,012	0,443	0,796	0,586

\* control of P. aeruginosa growth; \*\* MIC of single agent, \*\*\* MIC of the combination.

There was an antibacterial effect of betel extract started at the concentration of 150 mg/ml. Treatment with a combination of drugs has been used as an approach to overcome bacterial resistance, but the combination of betel extract with ciprofloxacin showed lower inhibition growth of P. aeruginosa compared than betel or ciprofloxacin alone. The addition of betel extract to ciprofloxacin didn't reduced the Minimum Inhibitory Concentration (MIC) of ciprofloxacin. The value of Fractional Inhibitory Concentration Index (FICI) is 3, there combination of betel extract and ciprofloxacin showed indifference interaction.

**Conclusion:** There was an indifferent antibacterial effect of the betel extract in combination with ciprofloxacin against P. aeruginosa. It isn't recommended to combine ciprofloxacin with betel on the infectious treatment of P. aeruginosa resistance to ciprofloxacin.

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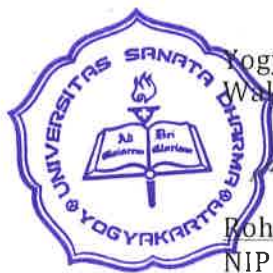
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**Letter of Acceptance**  
**2019 AASP Conference**

*“Impact of Rapidly Changing Environment on Pharmacy Education, Practice,  
and Pharmaceutical Sciences in Asia”*

Dear Yustina Sri Hartini,

We are pleased to inform you that your submitted abstract has been accepted for poster presentation at the 2019 Asian Association of Schools of Pharmacy (AASP) Conference which will be held on July 3 - 5, 2019 at Ajou University located in Suwon, Republic of Korea.

*Title:*

**Indifference Antibacterial Effect of Betel (Piper betle L.) Extract in Combination with  
Ciprofloxacin against Pseudomonas aeruginosa**

*Authors:*

**Yustina Sri Tri Hartini <sup>1</sup>**

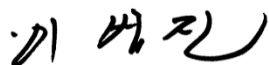
*Affiliations:*

**<sup>1</sup> Faculty of Pharmacy, Sanata Dharma University, Yogyakarta, INDONESIA**

The poster presentation schedule will be posted on the 2019 AASP Conference website (<http://aasp2019.org>). Should you have any further queries about the conference, feel free to contact me or 2019 AASP Conference Secretariat ([aasp2019secretariat@gmail.com](mailto:aasp2019secretariat@gmail.com)).

We appreciate your participation and look forward to seeing you in Suwon!

Sincerely yours,



Beom-Jin Lee, Ph.D.

Chair

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**[2019 AASP] Abstract Acceptance Letter**

Heain Hong &lt;aasp2019secretariat@gmail.com&gt;

Tue 4/30/2019 3:05 PM

To: Yustina Sri Hartini &lt;yustinahartini@usd.ac.id&gt;

 1 attachments (83 KB)

12. 2019 AASP\_Poster abstract acceptance\_Yustina Sri Hartini.pdf;

We are glad to inform you that your abstract has been peer reviewed and accepted by the **2019 Asian Association of Schools of Pharmacy (AASP) Conference**. This prestigious event will take place on **July 3 - 5, 2019** at Ajou University located in Suwon, Republic of Korea.

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We look forward to seeing you in Suwon!

Sincerely yours,

--

Heain Hong,

2019 Asian Association of Schools of Pharmacy Conference (AASP) Secretariat

Mobile: +82-10-7168-8441

Email: [aasp2019secretariat@gmail.com](mailto:aasp2019secretariat@gmail.com)

## Surat Pernyataan

Dengan ini saya :

Nama : Yustina Sri Hartini

Tempat, tanggal lahir : Sleman, 11 September 1969

NIDN : 0511096901

Dosen tetap pada Universitas Sanata Dharma

Menyatakan bahwa saya adalah peserta dan presenter poster pada **seminar internasional 9<sup>th</sup> Asian Association of Schools of Pharmacy (AASP)** yang dilaksanakan pada tanggal 3-5 Juli 2019 di Ajou University, Suwon Korea dengan judul naskah :

**Indifference Antibacterial Effect of Betel (*Piper betle* L.) Extract in Combination with Ciprofloxacin against *Pseudomonas aeruginosa***

Dengan jadwal presentasi pada tanggal 4 Juli 2019 jam 1:30 sampai 17:00 waktu Suwon, Korea.

Surat pernyataan ini saya buat sebagai bukti kehadiran karena panitia seminar tersebut tidak mengeluarkan sertifikat bagi peserta maupun presenter.

Demikian surat pernyataan ini saya buat, untuk digunakan sebagai bagian dari berkas pengajuan jabatan Guru Besar yang diajukan ke Kementerian Pendidikan Kebudayaan Riset dan Teknologi Republik Indonesia.

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Yang membuat pernyataan



(Yustina Sri Hartini)



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