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EFFECT OF ANTIBIOTIC IN PEPTIC ULCER THERAPY AGAINST
***Lactobacillus* sp. ISOLATED FROM FERMENTED MILK PRODUCT**
: IN VITRO STUDY

Rahardjo DN*, Pantjajani T, Kristin A***

* Faculty of Pharmacy, Surabaya University, Indonesia,

** Faculty of Technobiology, Surabaya University, Indonesia
dian_natasya@ymail.com

ABSTRACT

Background. The use of probiotics in conjunction with triple therapy in peptic ulcer disease may increase the eradication of *Helicobacter pylori*. It raises the question about the effect of antibiotic in triple therapy on the probiotic itself. Objectives. To determine the Minimum Inhibitory Concentration (MIC) of amoxycillin and metronidazole which used in triple therapy against *Lactobacillus* sp. isolated from fermented milk product. Method. Isolation of *Lactobacillus* sp. performed by pour plate method. The isolate of *Lactobacillus* sp. identified by macroscopic, microscopic observations and biochemical test. Broth dilution method was conducted to test the MIC of amoxycillin and metronidazole. Concentration of amoxycillin used were 25; 12.5; 6.25; 3.12; 1.56; 0.78; 0.39; 0.19; and 0.095 ppm. While the concentration of metronidazole were 5000; 2500; 1250; 625; 312.5; 156.25; 78.13; 39.06; 19.53 and 9.77 ppm. Result. The MIC of amoxycillin against *Lactobacillus* sp. was 0.19 ppm and the MIC of metronidazole was 5000 ppm. Conclusion. Amoxycillin may inhibit *Lactobacillus* sp. at levels lower than amoxycillin peak plasma levels and therefore can not be used simultaneously. Whereas the MIC of metronidazole against *Lactobacillus* sp. was greater than metronidazole peak plasma levels so that can be used simultaneously.

Key words: antibiotic, peptic ulcer, *Lactobacillus* sp., Minimum Inhibitory Concentration

INTRODUCTION

Helicobacter pylori plays an etiologic role in the development of peptic ulcer disease (Kumar, 2005). The standard therapy for peptic ulcer is a triple therapy consisting of a proton pump inhibitor and the antibiotics Clarithromycin and Metronidazole or Amoxicillin (Dipiro *et al.*, 2002).

However, study shows that the eradication rate of triple therapy is about 74-76%, therefore we need new treatment to improve the effectiveness of therapy (Brotoli *et al.*, 2007). Evidence suggests that ingesting lactic acid bacteria exerts a suppressive effect on *H. pylori* infection in both animals and humans. Supplementing with *Lactobacillus* was shown to inhibit *H. pylori* by interrupt its attachment to epithelial tissue, produce bacteriocins or antibiotic compound (Vilaichone *et al.*, 2002).

Consumption of probiotic products in conjunction with triple therapy shown to improve the therapeutic efficacy and reduce side effects caused by triple therapy (Lykova *et al.*, 1999).

Research shows pre-treatment for four weeks with antibiotic and yogurt can reduce the amount of *H.pylori* (Shyang, 2006). Meanwhile, other study mention that the use of triple therapy with probiotics together was more effective to improve the rate of eradication as much as 91% compared to the use of triple therapy alone, the eradication rate only 78% (Shyang, 2002).

The use of antibiotics and probiotics at the same time raises the possibility of interactions between antibiotics and probiotics, antibiotic may inhibit the probiotic agent. Amoxicillin is a beta-lactam antibiotic with a broad spectrum. Metronidazole is an antiprotozoa, but also it has bactericidal effect against anaerobic and

facultative anaerobic bacteria (Sweetman, 2009). Meanwhile, *Lactobacillus* sp., one of probiotics bacteria, is a gram-positive and microaerophilic.

Inhibition of probiotic agents can result in non-optimum treatment of peptic ulcer. Therefore, as a first step to recommend whether or not antibiotic and probiotic can be administered simultaneously, we need to test the inhibitory effect of antibiotic against *Lactobacillus* sp. and determination its MIC (Minimum Inhibitory Concentration).

The aim of this study is to determine the MIC of amoxicillin and metronidazole against *Lactobacillus* sp. isolated from fermented milk product "Y".

MATERIAL AND METHODS

Bacteria

Testing bacteria used in this study were *Lactobacillus* sp. isolated from the fermented milk product "Y". Samples obtained from the market in a fresh state. All samples were stored at 4-6°C prior to the isolation of *Lactobacillus* sp.

Antibiotics

Antibiotics used for MIC determination are amoxicillin and metronidazole.

Isolation of bacteria

Isolation was performed by pour plate method using specific media MRS (deMan, Rogosa and Sharpe) agar. Samples were incubated at 37° C for 24-48 hours to obtain pure cultures. Afterward, the colony has been observed and identified (Pelinescu *et al.*, 2008).

Identification of bacteria

Identification was performed by morphological observation, macroscopic, microscopic, Gram and spores staining, catalase test, and sugar fermentation test in liquid media (Leboffe & Pierce, 2008).

Minimum Inhibitory Concentration determination

Minimum inhibitory concentration (MIC) values was determined by the broth dilution method. The liquid medium used was MRS broth, since it is the specific medium for *Lactobacillus* sp. The MRS broth containing antibiotics at different concentrations. Concentration of amoxicillin used were 25; 12.5; 6.25; 3.12; 1.56; 0.78; 0.39; 0.19; and 0.095 ppm. While the concentration of metronidazole were 5000; 2500; 1250; 625; 312.5; 156.25; 78.13; 39.06; 19.53 and 9.77 ppm.

The inoculum was adjusted to a turbidity equivalent to 0.5 McFarland standard ($\approx 5 \times 10^5$ cfu/ml). The inoculum was derived from a broth culture which was incubated for 24 hour at 37 °C and 100 μ l of the inoculum was used to inoculate each tube.

Every tube were incubated at 37°C for 24 hour. The MIC was defined as the lowest concentration of antibiotic giving a complete inhibition of visible growth in comparison to an antibiotic-free control tube. To help the visual observation, an indicator p-iodonitrotetrazolium (INT) was added to each tube after incubation time, the color changing into red indicate the growth of bacteria. The experiments were replicated at least three times to verify the methodology reproducibility when using the above-mentioned conditions.

RESULTS AND DISCUSSION

Through the isolation and identification, the bacteria tested were *Lactobacillus* sp. with specific colony, small, white to yellow in color, rounded shape, raised elevation (Soeharsono, 2010). Microscopic observation showed bacil, paired, chain

with square-shaped ends, Gram-positive, and spore negative. Bacteria can ferment maltose, dextrose, and sucrose, and catalase negative.

We use MRS broth and MRS agar in isolation and MIC determination because it is a selective medium developed by de Man, Rogosa, and Sharpe that contain essential nutrients for growth of *Lactobacillus* sp. Ammonium citrate at low pH can inhibit most microorganisms but can grow *Lactobacillus*, dikalium phosphate and sodium acetate buffer compound to maintain a low pH, Tween 80 as emulsifier, manganese and MgSO₄ as a source of ions and sulfate, peptone and meat extract containing nitrogen, vitamins, minerals and amino acids essential for growth of *Lactobacillus*, and yeast extract as a source of vitamins (Merck, 2005).

The selection of antibiotic concentration in MIC determination based on the peak plasma of each antibiotic. The peak plasma of amoxicillin was 5 µg/ml (Sweetman, 2009), therefore the testing concentration were about 5 µg/ml. The peak plasma of metronidazole were 6-12 µg/ml (Sweetman, 2009), but because of its range was not show inhibitory effect, the testing concentration was increased.

The minimum inhibitory concentration (MIC) is the lowest antibiotic concentration that inhibits the visible bacterial growth after overnight incubation (Phillips *et al.*, 1991). The MIC of amoxicillin against *Lactobacillus* sp. was 0.19 ppm and the MIC of metronidazole was 5000 ppm. Due to the observation that bacteria develop antibiotic resistance, it is considered that when MICs are ≥8 µg/ml the bacteria may be considered as "moderately resistant"; when MICs are above 32 µg/ml it may be classified as "clinically resistant" to the antibiotic (Walsh, 2003). Therefore, this study show that *Lactobacillus* sp. isolated from fermented milk product "Y" is sensitive to amoxicillin and clinically resistant to metronidazole. The MIC of amoxicillin was lower than the peak plasma level, therefore amoxicillin and *Lactobacillus* sp. can not be used simultaneously.

Amoxicillin can inhibit the growth of *Lactobacillus* sp. because of its broad spectrum activity. The mechanism of action of amoxicillin is inhibition cell wall synthesis. Metronidazole works by selectively inhibit the synthesis of nucleic acids from anaerobic bacteria, possibly by act as a specific electron acceptor for reduced ferredoxin. This action will interfere with the electron transfer fosforoclastic reaction that can only be found in anaerobic and microaerophilic bacteria. The occurrence of resistance to metronidazole due to suspected bacterial pass the fosforoclastic reaction with other metabolic pathways or block the inhibition of electron transfer by metronidazole (Bayer *et al.*, 1978).

CONCLUSION

This study shows that the MIC of amoxicillin was lower than the peak plasma level, therefore amoxicillin and *Lactobacillus* sp. can not be used simultaneously. Whereas the MIC of metronidazole against *Lactobacillus* sp. was greater than metronidazole peak plasma levels so that can be used simultaneously.

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PROCEEDING

THE 3rd INTERNATIONAL CONFERENCE ON PHARMACY AND ADVANCED PHARMACEUTICAL SCIENCES

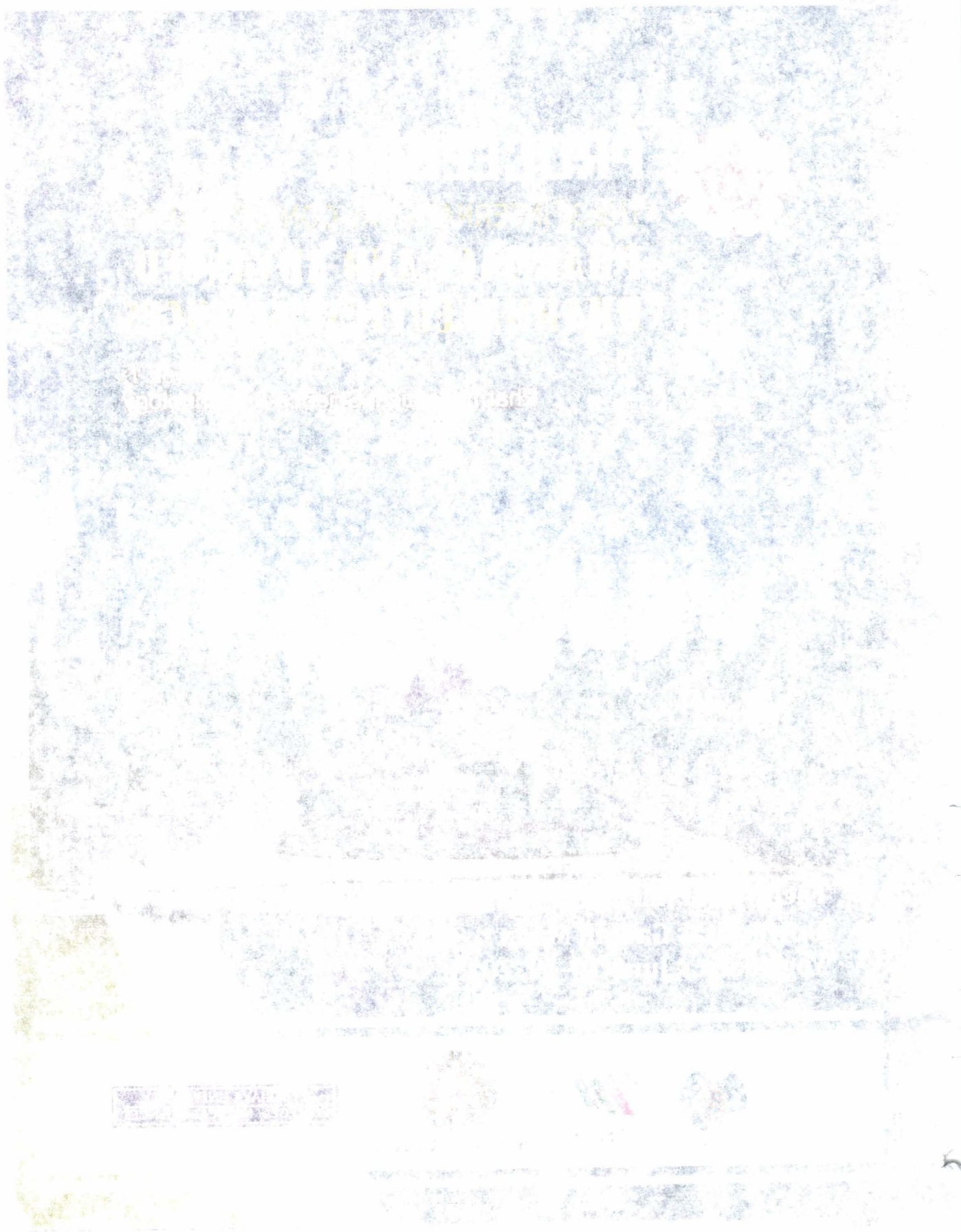
Book 1:
Pharmaceutical Science & Technology

Faculty of Pharmacy
Universitas Gadjah Mada
Yogyakarta, June 18-19, 2013



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PROCEEDING

The 3rd International Conference on
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Preface From Editor

On behalf of the Editors, I am deeply grateful to all the reviewers who have been working very hard for reviewing manuscripts submitted during the "3rd International Conference on Pharmacy and Advanced Pharmaceutical Sciences" held in Sheraton Hotel Yogyakarta, by the Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia on 18 - 19 June 2013.

We would like to acknowledge to keynote speakers and all the distinguished speakers for their valuable contribution during this conference. Furthermore, we also thank the steering committee for their advice and support. Finally, I would appreciate to all participants, paper and poster presenters who participated in the conference as well as cordially contributed by submitting their full manuscripts published in this proceeding.

Finally, we believe that the presence of this proceeding will significantly contribute to the advance scientific research, especially in the field of Pharmaceutical Science and Thecnology.

Yogyakarta, June 2013,
Chief

Abdul Rohman

Welcome to Yogyakarta

Assalamu'alaikum wr wb

Honorable Rector of Universitas Gadjah Mada, Prof. Dr. Praktino, M.Soc.

Honorable our keynote speaker : dr. Boenjamin Setiawan, PhD

Honorable our distinguished invited speakers, our guests, and all participants

First of all, let us praise to the Almighty Allah SWT, because of His Blessing we are able to attend this opening ceremony of the International Conference on Pharmacy and advanced pharmaceutical sciences today.

This morning, it is a great honor for me to welcome you all in this room in our conference. Welcome to Yogyakarta, and we hope you will enjoy your time here. This conference is the third international conference conducted by Faculty of Pharmacy Universitas Gadjah Mada to facilitate the experts meeting and sharing the knowldege among the researchers, academia, college students, policy makers in corresponding fields, and practitioners.

This year, the theme of The 3rd International Conference on Pharmacy and Advanced Pharmaceutical Sciences (ICPAPS 2013) is : **"Pharmaceutical development towards a sustainable and healthy society"**. The conference is conducted in collaboration with Utrecht University the Netherland, Nara Institute of Technology Japan, Mahidol University Thailand, and Cyberjaya University, Malaysia. Thank you very much for our international partners.

As a key note speaker in this conference, we are fortunate to have dr. Boenjamin Setiawan, PhD. He is the founder of Kalbe Farma, one of the big pharmaceutical company in Indonesia. His experience in developing pharmaceutical company as a bussinessman as well as his vision in development of medical and pharmaceutical research in Indonesia will be very inspiring, and hopefully will guide us to develop research in our respective fields. We also invited 12 more experts in various field of pharmacy and pharmaceutical sciences, either from Indonesia or overseas, who will give their lectures.

Here, among 300 participants, there are 175 presenters from 10 countries will present their recent research finding, which are divide into two big topics, Pharmaceutical Science and Technology and Clinical and Social Pharmacy. Our high appreciation and sincere gratitude are delivered to all speakers and presenters who enthusiastically participate in our conference.

The organizing commitee deeply acknowledges The Rector of Universitas Gadjah Mada, Nara Institute of Technology Japan, Mahidol University Thailand, and Cyberjaya University, Malaysia, as well as the sponsors for nice collaboration in conducting the conference. As the chairman of the committee, I personally would like to express our high appreciation and gratitude to all team members for the hard work, dedication, and invaluable efforts for the success of the conference.

Finally, we do hope that all participants could get benefit from this event and have enjoyable moment in Yogyakarta.

Wassalamu'alaikum wr wb.

Chairman

Zullies Ikawati

Remark

Dean, Faculty of Pharmacy, Gadjah Mada University

Firstly, let's thanks to Allah who always blesses to all of us, so that we can get together in this wonderful meeting, the 3rd International Conference on Pharmacy and Advanced Pharmaceutical Sciences (ICPAPS 2013). The Faculty of Pharmacy, Gadjah Mada University (GMU) is very happy to welcome all of you ICPAPS 2013 participants in the meeting and also we welcome all of you in Yogyakarta-Indonesia, the home of the Faculty of Pharmacy GMU.

Secondly, we'd like to give a brief introduction of our institution. Faculty of Pharmacy GMU was erected in September 1946, a year after Indonesian Independence, is noted as the oldest Faculty of Pharmacy in Indonesia. Faculty of Pharmacy GMU has been accredited nationally and internationally as well. In addition, collaboration in research, education, social services with several National and overseas Institutions have been established, intended to achieve our goals, one of those is quality of education. Recently, new regulations on Pharmacist roles in Indonesia have been emphasized as health profession, health promoter and pharmaceutical care. Therefore, theme of this ICPAPS 2013 meeting is selected as 'Pharmaceutical developments towards a sustainable and healthy society' that is parallel to those regulations. Faculty of Pharmacy GMU really hopes that this meeting is fruitful for all participants in general and specifically Pharmacy Institutions to develop to improve their education system.

Finally, Faculty of Pharmacy GMU highly appreciates to the Keynote speakers, invited speakers, all participants for spending your time with us, the Committee [Steering Committee, International partners (Universiteit Utrecht-The Netherland, NAIST-Japan, Mahidol University-Thailand, Cyberjaya University-Malaysia) Organizing Committee] who have been working very hard, and last but not least Faculty of Pharmacy GMU thanks to our sponsors for this meeting. Faculty of Pharmacy GMU realizes that without your participation, this meeting never happens.

Sincerely,

Subagus Wahyuono

Rector Speech

Rector, Universitas Gadjah Mada

It gives me genuine pleasure that Universitas Gadjah Mada has the honor of hosting the International Conference on Pharmacy and Advanced Pharmaceutical Sciences, which this year is in its third installment. This Conference, held in collaboration with Japan's Nara Institute of Sciences and Technology, the Netherlands' Universiteit Utrecht, Thailand's Mahidol University and Malaysia's Cyberjaya University, reflects a global commitment maintained by members of prominent think tanks the world over in addressing issues of common concern, which in this year's ICPAPS are Advanced Pharmaceutical Science and Social and Clinical Pharmacy.

Universitas Gadjah Mada, as a leading institution of higher learning in Indonesia, and in its commitment at becoming a World-Class Research University, has long since realized the spinal role of global cooperation in achieving our visions. This is why we welcome any and all effort through which international exchanges of thoughts and expertise can be encouraged. I see this perspective reflected in its entirety in ICPAPS, wherein experts and thinkers from all around the world will gather together and talk of a concerted effort to enhance the quality of pharmacists worldwide, broaden the insights on pharmacy as well as pharmaceutical sciences and technologies, and finally create both a national and international networking system for the dissemination of developments in pharmaceutical sciences and technologies.

Since those are very noble causes we all need to not only address, but also eventually produce into reality, I cannot impress the importance of this Conference for everyone present. Therefore, I can only hope that all the participants, be they researchers, academicians, pharmacists in and outside hospitals, as well as the students, are determined in making the best out of this brief gathering.

I am looking forward to seeing rigorous debates, heated discussions and, most importantly, a result that represents a joint international action held together by scientific truth. Thank you for all your commitment and dedication in organizing and contributing to this conference, and I wish you all the very best of luck.

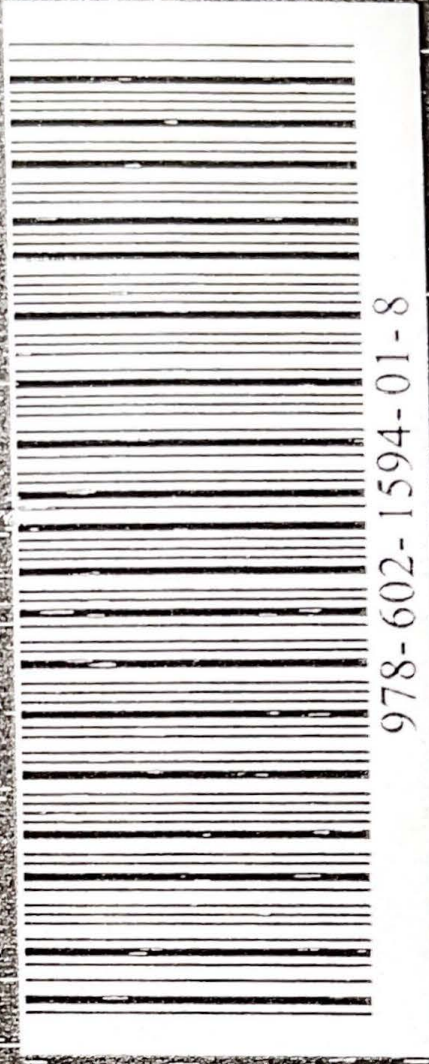
Prof. Dr. Pratikno, M.Soc. Sc.

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