ผลของสารสกัดแอลกอฮอล์จากเปลือกตะเคียนต่อ *mecA* POSITIVE *STAPHYLOCOCCUS AUREUS* ที่ดื้อต่อ METHICILIIN

นางสาว วิภาสินี วรรณศักดิ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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EFFECTS OF *HOPEA ODORATA* ROXB ALCOHOLIC EXTRACTS ON *mecA* POSITIVE METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

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Thesis Title	EFFECTS OF *Hopea odorata* ROXB. ALCOHOLIC EXTRACTS ON *mecA* POSITIVE METHICILLIN RESISTANT *Staphylococcus aureus*
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วิภาสินี วรรณศักดิ์ : ผลของสารสกัดแอลกอฮอล์จากเปลือกตะเคียนต่อ *mecA* POSITIVE STAPHYLOCOCCUS AUREUS ที่ดื้อต่อ METHICILLIN. (EFFECTS OF *HOPEA ODORATA* ROXB ALCOHOLIC EXTRACTS ON *mecA* POSITIVE METICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*) อ.ที่ปรึกษา : รศ.ศิริภรณ์ ฟุ้งวิทยา, อ.ที่ปรึกษาร่วม : รศ.ดร. พิณทิพย์ พงษ์เพ็ชร, 125 หน้า

เชื้อ methicillin resistant staphylococcus aureus (MRSA) เป็นแบคทีเรียที่มีความสำคัญทางการแพทย์ เป็นสาเหตุของการติดเชื้อในโรงพยาบาลที่พบได้บ่อย และในปัจจุบันยังพบว่าเชื้อมีการปรับตัวในการดื้อต่อยาปฏิชีวนะได้อย่างต่อเนื่อง เชื้อมากกว่า 90% มีการสร้างเอนไซม์ beta-lactamase และมีการเปลี่ยนแปลง penicillin binding protein(PBP) เป็น PBP2a โดยมี *mecA* gene เป็นยีนที่ควบคุมการสร้าง PBP2a ทำให้เชื้อดื้อต่อยา methicillin และมีการดื้อต่อยาหลายชนิดพร้อมกัน ทำให้เกิดปัญหาในการเลือกใช้ยาในการรักษา จึงเกิดแนวคิดที่จะนำสมุนไพรมาใช้ร่วมกับการใช้ยาด้านจุลชีพเพื่อหวังผลเสริมฤทธิ์กันและลดอุบัติการเชื้อดื้อยา การวิจัยครั้งนี้ได้นำสารสกัดแอลกอฮอล์จากเปลือกตะเคียน ยา ampicillin และ ยา ampicillin/sulbactam มาศึกษาฤทธิ์ต้านเชื้อ MRSA จำนวน 30 สายพันธุ์ พบว่ามีค่า MIC_{50} เท่ากับ 125 µg/ml, 128 µg/ml, 32 µg/ml ตามลำดับ เชื้อ MRSA 29 ใน 30 สายพันธุ์มีการสร้างเอนไซม์ beta-lactamase และเชื้อทั้งหมดดื้อต่อ ampicillin ในระดับสูง (MIC อยู่ในช่วง 8-128 µg/ml) เมื่อประเมินฤทธิ์ร่วมระหว่างยาและสมุนไพรต่อเชื้อ MRSA ทั้ง 30 สายพันธุ์โดยวิธี checkerboard พบว่าสารสกัดแอลกอฮอล์จากเปลือกตะเคียนความเข้มข้น 1/2 MIC ร่วมกับ ampicillin ปรากฏผลเสริมฤทธิ์บางส่วน (Partial synergy) ต่อเชื้อ 22 สายพันธุ์ (73.33 %) และผลเพิ่มฤทธิ์กัน (Additve) ต่อเชื้อ 8 สายพันธุ์ (26.67%) เมื่อใช้สารสกัดแอลกอฮอล์จากเปลือกตะเคียนความเข้มข้น 1/2 MIC ร่วมกับ ampicillin/sulbactam ปรากฏผลเสริมฤทธิ์บางส่วน (Partial synergy) ต่อเชื้อ 18 สายพันธุ์ (60 %) และผลเพิ่มฤทธิ์กัน(Additve) ต่อเชื้อ 12 สายพันธุ์ (40%) จากนั้นนำเชื้อ MRSA ทั้ง 18 สายพันธุ์ (ที่พบการเสริมฤทธิ์บางส่วนของยาและสารสกัด) มาประเมินผลการฆ่าเชื้อโดยวิธี Time kill assay พบว่าสารสกัดแอลกอฮอล์จากเปลือกตะเคียนความเข้มข้น 1/2 MIC ร่วมกับ ampicillin ความเข้มข้น 1/4 MIC และ 1/8 MIC หรือ ampicillin/sulbactam ความเข้มข้น 1/4 MIC และ 1/8 MIC สามารถฆ่าเชื้อได้ 90% ตั้งแต่ 2-6 ชั่วโมง โดยจำนวนเชื้อที่ถูกฆ่า(BA_{24})มากกว่าอย่างมีนัยสำคัญทางสถิติ เมื่อเทียบกับการใช้ยาเดี่ยว จากผลการทดลองแสดงให้เห็นว่า สารสกัดจากเปลือกตะเคียน ร่วมกับ ampicillin หรือ ampicillin/sulbactam มีฤทธิ์ยับยั้งเชื้อได้ดีกว่ายาหรือสมุนไพรเดี่ยว และเมื่อเปรียบเทียบกับยาเดี่ยวพบว่าการรวมยาและสารสกัดสมุนไพรจะเพิ่มจำนวนเชื้อที่ถูกฆ่าและลดระยะเวลาที่ใช้ในการยับยั้งการเจริญเติบโตของเชื้อ ดังนั้นการนำสารสกัดแอลกอฮอล์จากเปลือกตะเคียนมาพัฒนาต่อเพื่อนำมาร่วมกับ ampicillin และ ampicillin/sulbactam อาจเป็นอีกทางเลือกเพื่อรักษาโรคติดเชื้อ MRSA ที่ดื้อต่อ ampicillin และ ampicillin/sulbactam

สาขาวิชา.....เภสัชวิทยา.....ลายมือชื่อนิสิต.....

ปีการศึกษา.....2550.....ลายมือชื่ออาจารย์ที่ปรึกษา.....

ลายมืออาจารย์ที่ปรึกษาร่วม.....

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WIPASINEE WANNASAK : EFFECTS OF *HOPEA ODORATA* ROXB ALCOHOLIC EXTRACTS ON *mecA* POSITIVE METICILLIN RESISTANT *STAPHYLOCOCUS AUREUS*. THESIS ADVISOR : ASSOC. PROF. SIRIPORN FUNGWITTHAYA, M.Sc., THESIS CO-ASVISOR : ASSOC. PROF. PINTIP PONGPECH, Ph.D., 125 pp

Methicillin resistant *Staphylococcus aureus* (MRSA) infection is a problem in hospitalized patients worldwide. Combination therapy is the alternative choice in the treatment of MRSA with the aim of decreasing the emergence of resistant strains and increasing the bacterial killing. MRSA often acquires multi-drug resistance and causes severe problems in clinical medicine. Therefore, the purpose of the present study is to determine the susceptibility of of *H.odorata* ROXB as compared to those of ampicillin and ampicillin/sulbactam against 30 MRSA isolates. The MIC_{90} of the alcoholic extract of *H.odorata* ROXB, ampicillin and ampicillin/sulbactam were 125 µg/ml, 128 µg/ml and 32 µg/ml, respectively. Twenty-nine out of 30 isolates were beta-lactamase positive. All of the tested isolate were highly resistant to ampicillin (MIC range 8-128 µg/ml). The activity of the extract in combination with ampicillin and ampicillin/sulbactam were determined by Checkerboard method. Combination 1/2 MIC of the extract with ampicillin showed partial synergy against 22 isolates (73.33%) and additive against 13 isolates (26.67 %). While the combination 1/2 MIC of the extract with ampicillin/sulbactam showed partial synergy against 18 isolates (60 %) and additive against 12 isolates (40 %). In the Time kill study using MRSA 18 isolates (partial synergy in checkerboard method), combination of the extract 1/2 MIC with ampicillin showed bacteriostatic activity (90% killing) at 2, 4, 6 hr. The number of bacteria killed by the combination of extract 1/2 MIC plus 1/8 MIC ampicillin or 1/4 MIC ampicillin [BA_{24} = 64.04, 43.77 log CFU/ml·h, respectively] were significantly higher than the number killed by ampicillin alone [BA_{24} = 27.45, 18.43 log CFU/ml·h, respectively] ($p<0.05$). The combination of the extract 1/2 MIC with ampicillin/sulbactam showed bacteriostatic activity(90% killing) at 2, 4, 6 hr. The number of bacteria killed by the combination of extract 1/2 MIC plus 1/8 MIC ampicillin/sulbactam or 1/4 MIC ampicillin/sulbactam [BA_{24} = 37.75, 45.76 log CFU/ml·h, respectively] were significantly higher than the number killed by ampicillin/sulbactam alone [BA_{24} = 17.64, 25.75 log CFU/ml·h, respectively] ($p<0.05$). The results suggested that antibacterial activity of the combination between the herbal extract plus ampicillin or plus ampicillin/sulbactam were higher than the antibacterial activity of each drugs or herbal extract. It is concluded that the combination of extract plus ampicillin and ampicillin/sulbactam could be promising alternatives in the treatment of infections due to MRSA that were resistant to ampicillin and ampicillin/sulbactam.

Field of study.....PHARMACOLOGY.....Student's signature.....WIPASINEE WA.....

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LIST OF ABBREVIATIONS

°C	= degree Celsius
AUBC	= Area under the baterial killing regrowth curves
AUC	= Area under the curve
BA24	= Bacteriolytic area of 24 hours
CFU	= Colony forming unit
et al.	= et alii (and other peoples)
g	= gram
H.odorata ROXB	= *Hopea odorata* ROXB
hr	= hour
L	= Liter
log	= Decimal logarithum
MBC	= Minimum bactericidal concentration
MHA	= Meuller-Hinton agar
MHB	= Meuller-Hinton broth
MIC	= Minimum inhibitory concentration
min	= minute
ml	= milliliter
mm	= millimeter
mol	= mole
MRSA	= Methicillin resistant Staphylococus aureus
NCCLS	= The National Committee for Clinical Laboratory Standards
NSS	= Normal saline solution
TSA	= Tryptic soy agar
S.aureus	= *Staphylococcus aureus*
µg	= microgram
µl	= microliter

CHAPTER I

INTRODUCTION

Staphylococcus aureus (*S. aureus*) has been a major cause of infections in humans for as long as we have historical records. Pathological changes consistent with staphylococcal osteomyelitis are known from Egyptian mummies and other remains of similar antiquity. *S. aureus* can cause food intoxication, pneumonia, bacteremia, impetigo, folliculitis and osteomyelitis in humans, and mastitis, arthritis and urinary tract infection in animals.

Many antibiotics were used to treat the patients which were infected by this organism. Penicillin is the first antibiotic that was introduced in 1941. Within a few years, most hospital isolates were resistant to penicillin. In the late 1950s, semisynthetic penicillin was discovered and led to the development of "penicillinase resistant penicillin" such as methicillin, naficillin, oxacillin as well as cephalosporin. Such penicillins were not inactivated by penicillinase, so they were widely used in the treatment of *S. aureus* infection. Ironically, methicillin resistant *S. aureus* (MRSA) was isolated from hospitalized patients at about the same time. The prevalence of MRSA progressively increased thereafter (Diekema et al., 2001). MRSA is now endemic, and even epidemic, in many US hospitals, long-term care facilities (Strausbaugh et al., 1996), and communities (Crum et al., 2006). In Thailand, the prevalence of MRSA increased as many parts of the world. The reportes in 2006 revealed that MRSA caused 5% of nosocomial infection and 41.5% of *S. aureus* infections were methicillin resistant strains (Mekviwattanawong et al., 2006; Danchaivijitr et al., 2007). Because MRSA do not resist to only β-lactam antibiotics, but they do also resist to macrolides, licosamides and ciprofloxacin. Thus, the cost of treatment is high and becomes the problem in our health care system.

As the concerning about the problem in the treatment of multiple drug resistant pathogens, the medicinal plant could probably be another best solution. Medicinal plants have long been prescribed in the traditional medicine and at present, many compounds have been extracted from medicinal plants and have been found to be active against some specific types of infectious disease. Over the last two decades, several resveratrol oligomers, the stilbene derivatives, have been isolated from Dipterocarpaceae plants. These compounds exhibit diverse biological activities

including antibacterial, antifungal, anti-inflammatory, cytotoxic, and HIV inhibitory activities.

Zgoda-Pols et al., reported that the resveratrol tetramers, hopeaphenol A and vaticaphenol A, which were isolated from stem bark of *Vatica oblongifolia* had moderate activity against MRSA and *Mycobacterium smegmatis*. (Zgoda-Pols et al., 2002) In addition, many Dipterocarpaceae plants are grown in Thailand and *Hopea odorata* ROXB. is the one of these plants. Therefore, the purpose of the present study is to determine the susceptibility of MRSA against alcoholic extract of *Hopea odorata* ROXB. by paper disk diffusion method. The MIC of the two antimicrobial agents (ampicillin and ampicillin/sulbactam) and of alcoholic extract of *Hopea odorata* ROXB. against all MRSA isolates were also determined by the standard agar dilution method. At the same time, the combined antibacterial activities of alcoholic extract of *Hopea odorata* ROXB. plus ampicillin or plus ampicillin/sulbactam against MRSA were also determined by the checkerboard and time kill method. The expected outcome of the study is the information on the antibacterial activities in different aspects of *Hopea odorata* ROXB. (single and combined with commonly used antibiotics). These will lead to further study to determine the active ingredients from this for the development of plant new drug.

CHAPTER II

LITERATURE REVIEWS

1. *Staphylococcus aureus* and Methicillin resistance *S. aureus*

Staphylococcus aureus (*S. aureus*) is a member of the staphylococcus genus which are gram-positive cocci (0.5 to 1.5 μm in diameter) that occurs singly and in pairs, tetrads, short chains, and irregular grapelike clusters. (Ogston, 1883) introduced the name "staphylococcus" (from the Greek staphylé, a "bunch of grapes") to describe "micrococci" responsible for inflammation and suppuration. Staphylococci are nonmotile, non–spore forming, usually catalase positive (meaning that they can produce the enzyme "catalase") and able to convert hydrogen peroxide (H_2O_2) to water and oxygen, which makes the catalase test useful to distinguish staphylococci from enterococci and streptococci. They are often uncapsulated or have a limited capsule. Most species are facultative anaerobes.

Staphylococcus aureus (*S. aureus*) is a ubiquitous colonizers of the skin and mucosa of vitually all animals including mammals and birds. It is also widespread among the primates but not restricted to them. It is a major cause of disease (mastitis) in bovine and ovine herds (Pascal et.al., 2003). *S. aureus* demonstrates a niche preference for the anterior nares, especially in adults. It can exist as a resident or a transient member of the normal flora. Nasal carrier rate can vary from 10% to 40% in both the community and the hospital environment. Chronic nasal carriage may put certain population at increased risk for infection, such as patients with recurring furunculosis and patients who are subject to medical procedures including chronic hemodialysis or peritoneal dialysis or undergoing surgery (von Eiff et al., 2001; Laupland et al., 2003).

S. aureus can infect other tissues when normal barriers have been breached (e.g. skin or mucosal lining). This leads to furuncles (boils) and carbuncles (a collection of furuncles). In infants, *S. aureus* infection can cause a severe disease Staphylococcal scalded skin syndrome (SSSS). *S. aureus* infections can be spread through contact with pus from an infected wound, skin-to-skin contact with an infected person by producing hyaluronidase that destroy tissues, and contact with objects such as towels, sheets, clothing, or athletic equipment used by an infected

person. Deeply situated *S. aureus* infections can be very severe. Prosthetic joints put a person at particular risk for septic arthritis, and staphylococcal endocarditis (infection of the heart valves) and pneumonia, which may be rapidly spread. Some strains of *S. aureus* produce toxic shock syndrome toxin, which are the causative agent for toxic shock syndrome. Some strains that produce an enterotoxin are the cause of staphylococcal food poisoning (Gerald et al., 2005).

Treatment of *S. aureus* infection can be treated with many class of antibiotic. But the mainstay treatment is the β-lactam antibiotics. Penicillin was accidentally discovered by Alexander Fleming in 1928 and was developed and introduced to the market for clinical use in 1941. Two years after that, Kirby isolated the penicillin resistant *S. aureus* as known "Penicillinase-producing *S. aureus*" (Kirby, 1944). The prevalence of penicillinase-producing strains of *S. aureus* within hospitals soon began to rise as penicillin became readily available after World War II.

Although penicillinase-producing strains were universally present in hospitals by the early 1950s, community isolates of *S. aureus* were considered to be largely penicillin susceptible. Penicillin continued to be recommended as an effective anti-staphylococcal agent as late as the early 1970s (Weinstein, 1975). However, the first comprehensive description and accurate assessment of the epidemiology of drug-resistant strains of S. aureus were published in 1969. Examination of more than 2,000 blood culture isolates of *S. aureus* received at the Statens Serum institute in Copenhegen during the year 1957 to 1966 for which detailed information on the origin of infection (hospital or community) was available, confirming a high prevalence of penicillin resistance (85% to 90%) for *S.aureus* hospital isolates. Somewhat unexpected was that penicillinase-producing strains were almost as common in the community, with 65% to 70% of isolates resistant to penicillin. By the 1970s, many studies reported high prevalence of penicillinase-producing strains regardless of location. (Jessen et al.,1969)

In the late 1950s, synthetic penicillin was discovered and led the development of penicillinase-stable β-lactams such as cephalosporins and methicillin. Ironically, the first methicillin resistant *S. aureus* (MRSA) was described at about the same time (Barber, 1961). The prevalence of MRSA progressively increased thereby after (Chambers, 2001). One survey of the National Infections Surveillance System reported that the hospital prevalence of MRSA increased from 2.1% in 1975 to 35% in 1991 (Paullilio et al., 1992). It is now as high 70% in certain centers, but great

geographic variations exists. In a survey from SENTRY Antimicrobial Surveillance Program (1997 to 1999), the MRSA prevalence varied as follows: western Pacific region, 46%; United States, 34.2%; Latin America, 34.9%; Europe, 26.3%; Canada, 5.7%. Methicillin resistance varied greatly among countries within a region. In western Pacific countries, percentage of MRSA ranged from 23.6% (Australia) to more than 70% in Japan and Hongkong. In European centers, these percentages varied from less than 2% in the Netherlands to 54.4% in Protugal (Diekema et al., 2001). The estimated number of MRSA related hospitalizations increased more than doubled from 1999 to 2005 (Klein E, 2007). Addition to hospital acquired MRSA infection, community-acquired MRSA infection now becomes a serious problem. It was isolated in 1993 in Australia (Udo et al., 1993). In 2002, community-acquired MRSA infection were reported between 8% and 20% of all MRSA isolates (Scott et al., 2005).

In Thailand, the prevalence of MRSA is increasing. Reported from many hospitals in 2006 revealed that the prevalence of MRSA infection was 5% of all nosocomial infection (Danchaivijitr et al., 2007). The *S. aureus* isolates from patients at Siriraj hospital, 41.5% were MRSA. The community-acquired MRSA infection is umcommon (Mekviwattanawong et al., 2006).

2. Treatment and antibiotic resistance

2.1 β -lactam antibiotics

β -lactam antibiotics, which contain β-lactam ring as a main structure, include penicillins, cephalosporins, carbapenems and monobactams.

2.1.1 **Penicillin**

Penicillin is a first antibiotic of β-lactam group. It used in the treatment of bacterial infections caused by susceptible, usually Gram-positive, organisms. "Penicillin" is also the informal name of a specific member of the penicillin group penam skeleton, which has the molecular formula R-C9H11N2O4S, where R is a variable side chain (Figure 2-1).

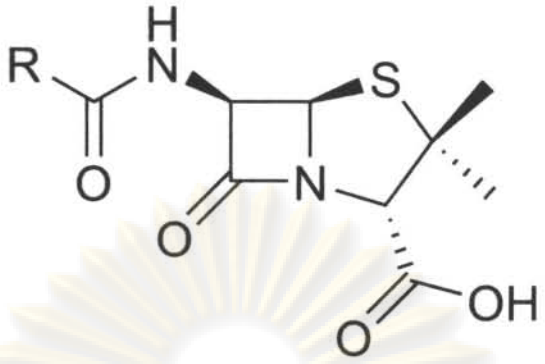


Figure 2-1: Chemical structure of penicillin core, R is a variable side chain

Mechanism of action

The antibacterial activity of penicillin is due to cell wall inhibition of bacterial cell wall synthesis. The cell wall of bacteria is a rigid structure of peptidoglycan that protects against osmotic rupture, especially in gram-positive bacteria. It assembled in a series of enzymatic steps involving at least 30 enzymes. The basic subunit of the peptidoglycan component is a disaccharide monomer of *N*-acetylglucosamine (NAG) and *N*-acetylmuramic (NAM) pentapeptide (Fig 2-2). These two disaccharides are composed of a long polysaccharide chain. The pentapeptide consists of amino acid residues alternating between L- and D-stereoisomers and terminating in D-alanyl-D-alanine. A stem peptide of variable length and composition is attached to the third amino acid of this pentapeptide. Pentapeptides are then joined with stem peptides to form a crosslink between polysaccharide chains. This reaction is catalyzed by a transpeptidase that forms an amide bond between the terminal-free amine group of a stem peptide and the penultimate D-alanine of a pentapeptide, displacing the terminal D-alanine in the process. This transpeptidation reaction is sensitive to inhibition by penicillin. There are distinct transpeptidases that provide for anchoring of new peptidoglycan to old, that cross link special structures, and that direct formation of the cell wall septum.

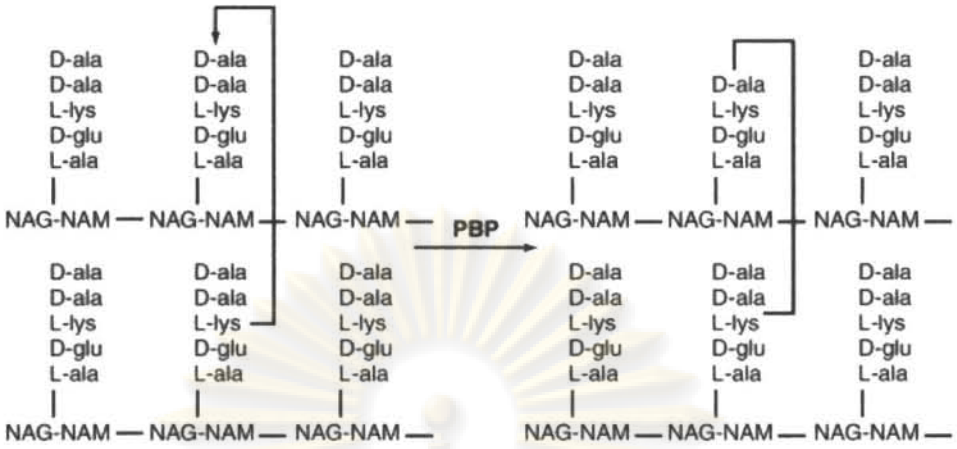


Figure 2-2: Penicillin-binding protein (PBP) transpeptidation reaction that cross links bacterial cell wall. NAG, *N*-acetylglucosamine; NAM, *N*-acetylmuramic.

The penicillin-sensitive reactions are catalyzed by a family of closely related proteins, penicillin-binding proteins (PBPs). Bacteria produce four type of PBPs, which structurally resemble and likely, are derived from serine proteases. High-molecular-weight PBPs (i.e., >50 kD) and low-molecular-weight PBPs catalyze transpeptidation and carboxypeptidation reactions of cell wall synthesis, respectively. Penicillin receptors PBPs transit a transmembrane signal for induction of β-lactamases (Krogstad and Pargwette, 1980). β-lactamases are PBPs that catalyze hydrolysis of the β-lactam ring. Except for β-lactamases, which may be either secreted or membrane associated, PBPs are membrane bound. β-lactam antibiotics inhibit cell transpeptidation step. They compete with the cell wall precursor for binding to the active site of the enzyme and undergo nucleophilic attack a their C=O residue in a similar manner to the PBP natural D-ala-D-ala substrate . However, unlike natural D-ala-D-ala, the β-lactam-PBP acyl adduct is stable, resulting irreversible blockage of PBP function. The bacteria cannot generate new cell wall cause decrease the cell wall intregity and lead to cell death.

2.1.2 **Aminopenicillins**

Aminopenicillins consist of ampicillin and amoxicillin (Figure 2-3). They are not stable to β-lactamases. For practical purposes, the activity of aminopenicillins is virtually identical to that of penicillin against penicillin-susceptible organisms, except that aminopenicillins are slightly more active against enterococci. Non–β-lactamase–

producing strains of *Haemophilus influenzae* and *Haemophilus parainfluenzae* are susceptible. Strains of *E. coli*, *Shigella sonnei*, and *Salmonella* spp., including many strains of *Salmonella typhi,* once uniformly susceptible to aminopenicillins, often are resistant due to β-lactamase production. *Klebsiella* spp., *Serratia, Acinetobacter*, indole-positive *Proteus*,*Pseudomonas* spp., and strains of *Bacteroides fragilis* are resistant to aminopenicillins.

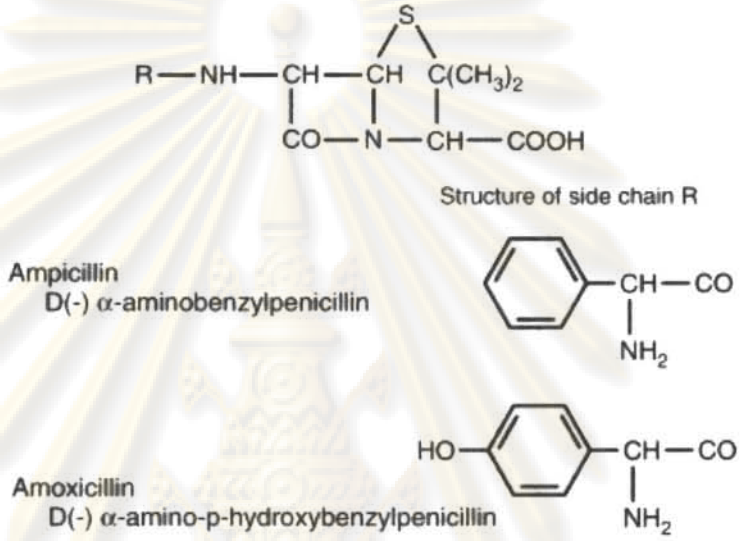


Figure 2-3: Chemical structure of Aminopenicillin

Mechanisms of Penicillin Resistance

Four mechanisms account for clinically significant bacterial resistance to penicillins, and other β-lactam antibiotics as well: (1) destruction of antibiotic by β-lactamase, (2) failure of antibiotic to penetrate the outer membrane of gram-negatives to reach PBP targets, (3) efflux of drug across the outer membrane of gram-negatives, and (4) low-affinity binding of antibiotic to target PBPs.

The most common mechanism of *S. aureus* resistance to β-lactams involves β-lactamase, which is encoded by the *bla* gene which is on a plasmid. The gene is inducible and preceded by the *blaRI* and *blaI* regulatory determinants. Penicillinase is a secreted enzyme that hydrolyzes penicillin and other penicillinase-susceptible compounds into inactive penicilloic acid (Ghuysen, 1994).

The minimal inhibitory concentration (MIC) of penicillin G for fully susceptible *S. aureus* is approximately 0.01 mg/L. In contrast, the MIC of penicillinase-stable drugs such as nafcillin or cephalosporins is 10-fold greater. Thus,

penicillin G remains one of the best choices against penicillin-susceptible staphylococci.

2.1.3 **Penicillinase-Resistant Penicillins**

The discovery that 6-aminopenicillanic acid could be obtained from cultures of *P. chrysogenum* that were depleted of side-chain precursors let to the development of the semisynthetic penicillins (Brewer and Johnson, 1953; Tosoni et al., 1958). Side chains can be added that alter the susceptibility of the resulting compounds to inactivating enzymes, β-lactamase, and that change the antimicrobial activity and the pharmacologic properties of the drug. The antibacterial spectra of all penicillinase-resistant penicillins are identical. They are active against methicillin-susceptible strains of staphylococci; penicillin-susceptible strains of streptococci, including *S. pneumoniae*; and most anaerobic gram-positive cocci. None are active against methicillin-resistant staphylococci, high-level penicillin-resistant streptococci, enterococci, *Listeria monocytogenes,* aerobic gram-negative cocci or bacilli, or anaerobic gram-negative bacteria.

Methicillin, the narrow spectrum beta-lactam and the first semisynthetic penicillin, was introduced by Beecham and Bristol in 1959 (Batchelor, 1959). The presence of the ortho-dimethoxyphenyl group directly attached to the side chain carbonyl group of the penicillin nucleus facilitates the β-lactamase resistance, since those enzymes are relatively intolerant of side-chain steric hindrance (Figure 2-4). Thus, it is able to bind to penicillin binding proteins (PBPs) and inhibit peptidoglycan crosslinking, but is not bound by or inactivated by β-lactamases. Methicillin is the least active of the penicillinase-resistant penicillins. It is acid-labile and therefore, can be administered only parenterally. It is more likely to cause interstitial nephritis than the other penicillinase-resistant penicillins. For these reasons, methicillin is no longer used clinically (Gerald et al., 2005).

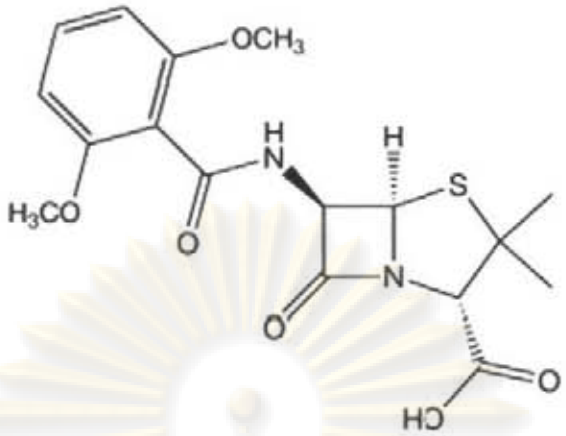


Figure 2-4: Chemical structure of methicillin

Nafcillin, Isoxazolyl penicillin (oxacillin, cloxacillin, and dicloxacillin) are others penicillinase-resistant penicillin which were developed later and widely used nowadays.

Mechanism of Methicillin Resistance

The main mechanism of methicillin resistance is mediated by the newly acquired PBP2A. As mention above, PBPs responsible for inserting the peptidoglycan precursors into the new wall (Ghuysen, 1994). Several of these PBPs are bifunctional and retain both a transglycosidase and a transpeptidase activity. *S. aureus* carries only one bifunctional PBP (PBP2) and three monofunctional transpeptidase (PBP1, 3 and 4).

All methicillin resistant strain carries a mobile genetic element SCC*mec*, where SCC stands for staphylococcal chromosomal cassette and *mec* for the gene encoding penicillin-binding protein (PBP) 2A. PBP2A has a low β-lactam affinity and confers resistance to most molecules of this family (Chambers et al., 1985; Tetsuro et.al., 2003). Thus, PBP2A can mediate cell wall assembly when the normal staphylococcal PBPs are blocked by these compounds (Figure 2-5) (De Jonge and Tomas, 1993). These make SCC*mec*-contained *S. aureus* resisted to β-lactam.

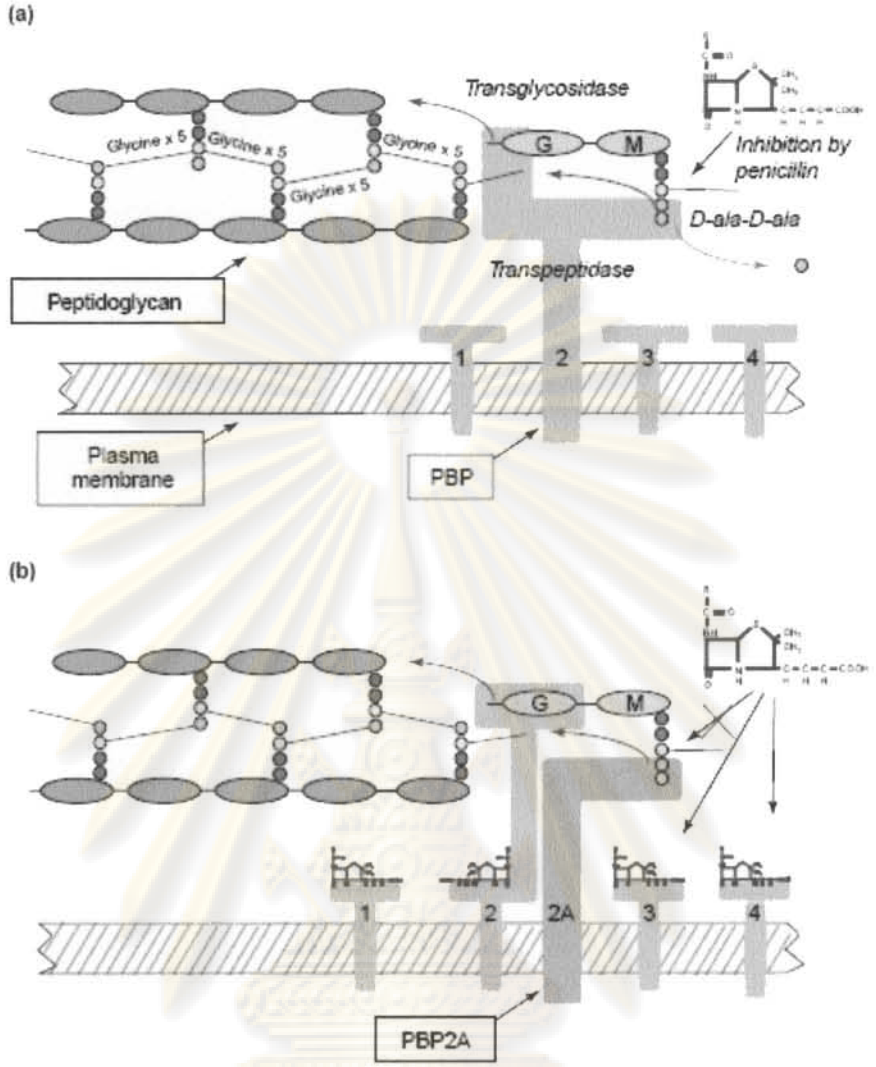


Figure 2-5: (a) Cell wall precursors comprise the disaccharide pentapeptides N-acetylglucosamine and *N*-acetyl-muramic acid-L-ala-D-glu-L-lys-D-ala-D-ala. After membrane translocation, the precursors are processed by membrane PBPs. High molecular weight PBPs are bifunctional enzymes that perform both a transglycosidase step, linking the incoming *N*-acetylglucosamine (G) to a muramate (M) in the nascent wall, and a transpeptidase step, linking the penultimate D-ala to a glycine acceptor in the new wall. In *S. aureus*, the lysine in position 3 of the stem peptide is almost always decorated with a pentaglycine side-chain. Penicillin is a mechanism-based inhibitor of the transpeptidase domain of PBPs. (b) MRSA carry an additional PBP called PBP2A, which has very low affinity for most available β-lactam drugs. Therefore, when β-lactams are present, they block the normal PBPs, but not PBP2A. PBP2A has only a transpeptidase domain (de Lencastre et al.,1999).

However, PBP2A has a special requirement for peculiar cell wall precursors. These must contain a pentaglycine-decorating side chain attached to the position 3 L-lysine of their stem peptide (Rohrer and Berger-Bachi, 2003), as well as other specificities such as an amidated D-glutamine in position 2 of the peptide (Figure 2-5). Providing this adequate substrate requires the functionality of numerous accessory genes implicated in the normal wall building machinery (B, 1994; de Lencastre H, 1994). These include more than 20 accessory determinants, some of which (*femAB* and *fmhB*) are responsible for adding the glycine residues critical for the PBP2A function (B, 1994). Any alteration in these elements decreases methicillin resistance despite the expression of PBP2A

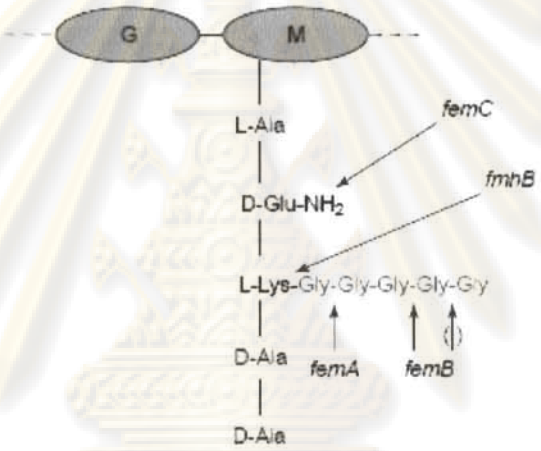
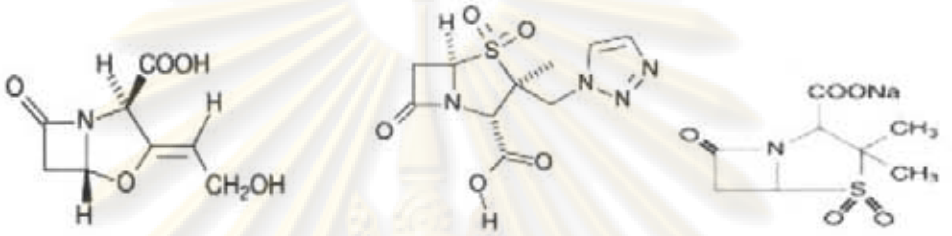


Figure 2-6: To be functional, PBP2A requires that the cell provides fully decorated precursors, containing both a pentaglycine side-chain and an amidated glutamine. Inactivation of the *femB, femA*, and *fmhB* genes blocks the addition of pentaglycines, and thus decreases the expression of methicillin resistance even though PBP2A is present in the bacterial membrane. Inactivation of femC has a similar effect (Gerald et al., 2005).

An additional fragility of PBP2A is that it carries only a transpeptidase domain, and has no transglycosidase activity (Figure 2-6). Thus, to successfully assemble the peptidoglycan it also need to use the tranglycosidase domain of normal PBP2 (Pincho et al., 2001). Taken together, the requirement for special precursors and the need of transglycosidase domain of normal PBP2 represent the weak point of the resistance system.

2.2 Role of β-Lactamase inhibitor

The β-lactamase inhibitors are structurally related to β-lactam antibiotics, retaining the amide bond of the β-lactam ring of the parent compound, but a modified side chain. These structural features enable the inhibitors to bind irreversibly as suicide substrates to the β-lactamases, rendering them inactive. There are three β-lactamase inhibitors currently used in clinical practice namely clavulanic acid, sulbactam and tazobactam(Figure2-7)



Clavulanic acid Tazobactam sulbactam

Figure 2-7: Molecular structres of β-lactamase inhibitor

β-lactamase inhibitors are not only able to inhibit the β-lactamase capacity, but they also exhibit β-lactamase induction effect notably AmpC that is β-lactamase categorized in cephalosporinase group. Thus, the medical team hould carefully practice the BL-BI combinations. Nowadays, there are five currently available BL-BI combination, which are drug of choice for the treatment infectious diseases caused by β-lactamase producing bacteria. (as shown in table 2-1)

Table 2-1 : β-lactam-β-lactamase inhibitors for clinical use

β-lactam	β-lactamase inhibitors	Administration route	Combination(mg) (BL:BI)
Ampicillin	Sulbactam	Parenteral and oral	1000:500
Cefoperazone	Sulbactam	Parenteral only (not available in the USA)	2000:1000 500:500(Thailand)
Piperacillin	Tazobactam	Parenteral only	4000:500
Ticarcillin	Clavulanic acid	Parenteral only	3000:100
Amoxicillin	Clavulanic acid	Parenteral and oral (only oral form available in the USA)	1000:200, 500:100 250:125, 500:125

BL = β-lactam, BI = β-lactamase inhibitor

Protection of a labile β-lactamase inhibitor provides an alternative strategy for overcoming β-lactamases. The combination of ampicillin and oxacillin were occasionally used against *P. aeruginosa* urinary tract infection as early as 1963, based on the reasoning that oxacillin should inhibit the organism's AmpC enzyme, which otherwise destroys the ampicillin. This combination was not very effective, propably because oxacillin penetrates *P. aeruginosa* poorly or is pumped out, and the strategy was dropped with the development of carbenicillin. Interest reawakened in the mid-1970s, when several class of inhibitor were found in rapid succession, including clavulanic acid, penicillanic acid sulphones, halogenated penicillanic acid sulphones have been developed into clinical used in the current.

Clavulanic acid was destined to become the first β-lactamase inhibitor to enter clinical use. The discovery of clavulanic acid futher stimuiated the search for other β-lactamase inhibitors, and eventually led to the development of the penicillanic acid sulphones, sulbactam, and tazobactam, which are now available clinically. Each inhibitor is available only as a fixed-combination preparation that includes an active β-lactam antibiotic as the companion agent. There are minor differences in potency, and pharmacology among the β-lactamase inhibitors, and clinically they can be considered therapeutically equivalent. The antibacterial activity of the β-lactam-β-lactamase inhibitor combination is determined by the spectrum of the companion β-lactam antibiotic.

Type of β-lactamase inhibitors

1. Clavulanic acid

Clavulanic acid is naturally occurring weak antimicrobial agent found initially in cultures of *Streptomyces clavuligerus.* This agent acts primarily as a "suicide inhibitor" by forming an irreversible acyi enzyme complex with the β-lactamase, leading to loss of activity of the enzyme. Clavulanic acid acts synergistically with various penicillins and cephalosporins against β-lactamase-producing gram negative bacteria. Currently, clavulanic acid is available for clinical use in a 1:2 and 1:4 combination with oral amoxicillin and in a 1:15 and 1:30 parenteral combination with ticarcillin. The pharmacologic parameters of amoxicillin and ticarcillin are not significantly altered when the drug is combined wih clavulanic acid. Amoxicillin-clavulanate is moderately well absorbes from the gastrointestinal tract, with a half-life

in serum of about 1 hr. for each component. One-third of a dose is metabolized, while the remainder is excreted unchanged in the urine. The drug is widely distributed to varius body tissue and fluids, but it penetrates uninflamed meninges very poorly.

2. Sulbactam

Sulbactam is semisynthetic 6-desaminopenicillin sulfone with weak antibacterial activity. It acts synergistically with penicillins and cephalosporins against β-lactamase-producing gram negative bacteria. For clinical use, sulbactam is combined with ampicillin and cefoperazone as a parenteral preparation in a 1:2 ratio and 1:1, 1:2 ratio, respectively. The pharmacologic properties of the drugs are not affected by each other in these combinations. Ampicillin-sulbactam penetrates well into body tissue and fluids, including peritoneal and blister fluids. It enters the CSF in the presence of impaired renal function, dosage adjustments are similar for the two drugs.

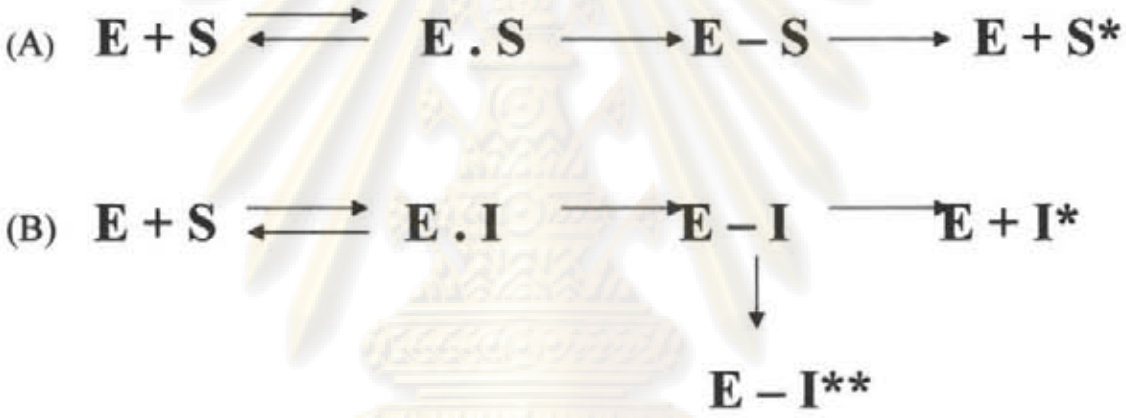
3. Tazobactam

Tozobatam is a penicillanic acid sulphone derivertive structurally related to sulbactam. Like clavulanic acid and sulbactam, tazobactam acts as a suicidal β-lactamase inhibitor and binds to bacterial PBP1 or PBP2. Despite having very poor intrinsic antibacterial activity by itself, it is comparable to clavulanate and subactam in lowering the MICs by to 20-fold for many organisms when combined with various β-lactam against β-lactamase-producing organisms. Of the penicillin-β-lactamase inhibitor combinations, piperacillin-tozabactam is the most active (twofold to eightfold lower MICs) against β-lactamase-producing aerobic and anaerobic gram negative bacilli. Tazobactam is administered parenterally as a 1:8 combination with piperacillin. The two drugs do not affect each other's metabolism or pharmacokinetics. High concentrations of both agents are achieved in the intestinal mucosa, lungs, and skin, with relatively poor distribution to muscle, fat, prostate, and CSF (in the absence of inflamed meninges). With a half-life in serum of about 1 hr, tazobactam is eliminated mainly via the renal route and is not affected by hepatic failure.

Mechanism of β-lactamase inhibitors

The means by which clavulanic acid, sulbactam and tazobactam fuction as inhibitors of bacterial β-lactamases has been studied in detail with active-site serine β-lactamase. The data show similarities in the modes of action of the three agents and may be regarded as irreversible, suicide inhibitors of target enzyme.

Most clinically important β-lactamase have a serine hydroxyl group at the active site, which forms a non-covalent complex with β-lactam-carbonyl bond of the β-lactam substrate. An acylation reaction follows with the formation of an acyl-enzyme and opening of the β-lactam ring. In the case of β-lactamase-labile antibiotics the acyl-enzyme complex hydrolyzes rapidly to liberate free enzyme and the antibacterial inactive product (Figure 2-8A). In the case of suicide inhibitor the acyl-enzyme intermediate is comparatively stable and may react slowly to yield hydrolyses inhibitor and reactived enzyme or achieve stability by further reaction with enzyme(Figure 2-8B). Such reactions are possible because the hydrolysis of β-lactam moiety of clavulanic acid or the penicillanic acid sulphones unmasks reactive groups that can form stable covalent bonds at the active site.



(Modified form Sutherland, 1995)

Figure 2-8 Models for inhibition of β-lactamase with (A) β-lactamase-labile substrate(penicillin), and (B) with a β-lactamase suicide inhibitor ; E = enzyme ; S = substrate ; I = inhibitor ; E.S = noncovalent complex ; E–S = acyl-enzyme complex ; S* = hydrolyzed (inactive product) ; E-I** = permanently inactived enzyme ; I* = hydrolyzed inhibitor

Spectrum of inhibition of β-lactamase

β-lactamase inhibitors are most effective against β-lactamases produced by *S.aureus*, *H.influenzae*, *M.caterrhalis*, *Bacteroided* spp., and some Enterobacteriaceae Chromosomal β-lactamase of Serratia spp., C.freundii, Enterobacter spp., P.aeruginosa, but some Enterobacteriaceae are not inhibited by β-lactamase inhibitors.

3. Medicinal plant

The history of medicinal plants dates back to the origin of human civilization on earth. They have been widely used to treat a variety of infectious and non-infectious aliments. According to one estimate, 25% of the commonly used medicines contain compounds isolated from plants. Several plants could offer a rich reserve for drug discovery of infectious diseases, particularly in an era when the latest separation techniques are available on one hand, and the human population is challenged by a various infectious diseases on the other hand. Although the emerging infectious diseases are the challenged problem in health care system, but the multiple drug resistant infections are the serious problem too. Medicinal plant is another solution to deal with the multiple drug resistant organisms

Dipterocarpaceae is a large family of 17 genera and approximately 500 species of mainly tropical lowland rainforest trees. Various chemical compounds were extracted from plant of Dipterocarpaceae family, mostly are phenolic compounds. Many stilbene derivertives, the phenolic compounds, were extracted from Dipterocarpaceae plants, such as hopeaphenol, balanocarpol, copalliferols, stemonoporol, vaticaffinol (Subramaniam and Vinayagar, 1993), vaticanol A, B and C (Tanaka et al., 2000), pauciflorols A, B and C, isovaticanols B and C, pauciflorosides A, B and C (Tetsuro et al., 2003), cotylelosides A, B and C, vaticaside A, B, C and D (Tetsuro et al., 2006), vateriaphenol A and B (Tetsuro et al., 2003), laevifonol, hemslevanols A and B (Tukirana et al., 2005).

Many studies of stibene derivetives revealed that they had antibiotic effect, antiviral effect and antitumor effect. Zgoda-pols et al. reported the resveratrol tetramer, hopeaphenol A, isohopeaphenol A (Figure 6a,b) and vaticaphenol A, that extracted from the stem bark of *Vatica oblongifolia* spp. oblongifolia. Antimicrobial testing on these compounds was performed. The minimum inhibitory concentrations (MICs) of hopeaphenol A and vaticaphenol A were determined to be 100 and 50 μg/ml against MRSA, respectively. Isohopeaphenol A was inactive against MRSA (MIC > 100 μg/ml) (Zgoda-Pols et al., 2002).

Hopea is one of genus in Dipterocarpaceae family. Many stilbene derivatives were extracted from the stem bark of *Hopea parviflora* such as parviflorol, ampelosin balanocarpol, ε-viniferin and hopeaphenol(Tanaka et al., 2000). Thus, *Hopea odorata* ROXB. should contain these compounds too.

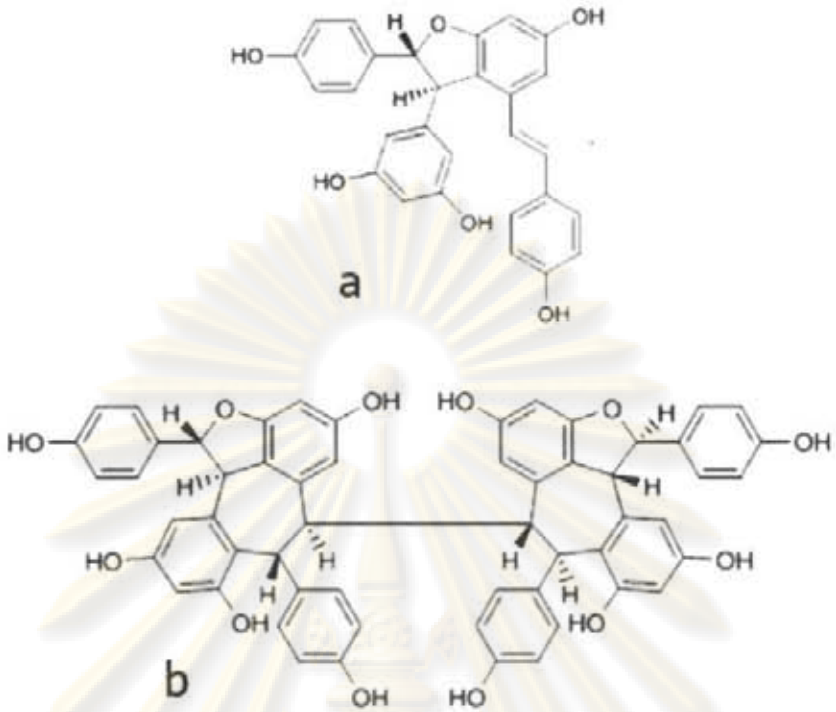


Figure 2-9: Chemical structure of hopeaphenol (a) and isohopeaphenol (b)

Hopea odorata ROXB. is a member of genus Dipterocapaceae, The other botanic name is *Hopea eglandulosa* ROXB. *Hopea odorata* ROXB. has many common names, such as sauchi, thingan net in Burmese, white thingan in English, merawan siput jantan in Malay, koki mosau, sao den in Vietnamese and trade name is thingan or white thingan, Takian. Takian Thong is a common name of *H.odorata* Roxb. in Thai.

Hopea odorata ROXB is a medium-sized to large evergreen tree with a large crown growing to 45 m. tall, bole straight, cylindrical, branchless to 25 m, with diameter of up to 4.5 m. or more and prominent buttresses, bark surface scaly, grey to dark brown, longitudinally furrowed, yellow or reddish inside. Leaves ovate-lanceolate, 7-14 by 3-7 cm, falcate, base broadly cuneate, venation scalariform, midrib applanate to slightly channeled above, glabrous on both surfaces, petiole 2 cm long, slender. Flowers small, sweet scented, yellowish-white, very shortly pedicelled, in one-sided racemes, stamens 15, anthers narrowly ellipsoid, ovary ovoid, punctate or glabrous. Fruit small, ovoid, wings oblanceolate, rounded, 3-4 cm long, finely veined lengthwise. The specific epithet means odour and refers to the sweet smell of the flowers.

Hopea odorata ROXB is a riparian species usually occurring on deep rich soils, most commonly along the banks of streams and in damp situations up to 600-m altitude. It is chiefly found in the tropical forests of Bangladesh, Cambodia, China, India, Laos, Malaysia, Myanmar, Vietnam, and Thailand (Soerianegara and Lemmens , 1993).

Figure 2- 10 *Hopea odorata* ROXB.

4. Combination therapy

Antibiotics are frequently used in combination for treat a life-threatening infection, prevent emergence of bacterial resistance, treat mixed infections of aerobic and anaerobic bacteria, enhance antibacterial activity (synergy) and use lower doses of a toxic drug. Combined treatment is reasonable when the precise agents of a serious infection are unknown. Use of two or more drugs to prevent the emergence of resistance is effective for therapy of some infections.

Penicillin shows a synergistic effect with aminoglycosides, since the inhibition of peptidoglycan synthesis allows aminoglycosides to penetrate the bacterial cell wall more easily, allowing its disruption of bacterial protein synthesis within the cell. This results in a lowered the minimum bactericidal concentration (MBC) for susceptible organisms. This combination is the effective treatment of native valve endocarditis caused by *S. aureus* (Chambers, 1993).

As above, *S. aureus* produce β-lactamase which hydrolyze penicillin to inactive penicilloic acid. So, the β-lactamase inhibitors were developed to block the function of β-lactamase and restore the antibacterial activity of β-lactam antibiotics.

β-Lactamase inhibitors are clavulanic acid and penicillanic acid sulfone derivatives. These compounds, which have weak antibacterial activity, are potent inhibitors of many plasmid-encoded and some chromosomal β-lactamases.

Clavulanate is one of the β-lactamase inhibitors. It was found in cultures of Streptomyces clavuligerus. Clavulanate subsequently was found to inhibit certain types of β-lactamases from many clinically important gram-positive and gram-negative organisms. When combine with amoxicillin, they are the choice for treatment of otitis media, sinusitis, bronchitis, urinary tract infection and skin and soft tissue infections.

Sulbactam, another β-lactamase inhibitor, is a 6-desaminopenicillin sulfone. Sulbactam has a broader spectrum β-lactamase inhibitor than clavulanate, but less potent. In vitro study, combination of sulbactam and ampicillin can inhibit growth of *Streptococcus, Haemophilus, Neisseria, Branhamella, Bacteroides, Escherichia coli, Klebsiella, Enterobacter aerogenes, Proteus, and Acinetobacter calcoaceticus* (Retsema et al., 1986).

The combination therapy for treatment MRSA infection were widely studied. Vancomycin is almost universally accepted as the drug of choice for the treatment of MRSA infections. However, vancomycin used alone kills staphylococci slowly, resulting in delayed recovery of patients with life-threatening infections. In addition, the clinicians now have to face the emergence of strains with reduced susceptibility to vancomycin. Therefore, the combination therapy are widely studied to optimized the best treatment for MRSA infection.

Treatment with MRSA infection with vancomycin can reduced the drug susceptibility and under selective pressure. Meanwhile, in vitro experiments have demonstrated that selective pressure can produce vancomycin resistance but have also revealed that increase in vancomycin resistance can induce concurrent decrease in resistance to β-lactams in MRSA. Domaracki et al. reported the clinical isolates of vancomycin-susceptible MRSA become increasingly susceptible to oxacillin when grown in the presence of a sub-MIC of vancomycin. In addition, checkerboard assays and time-kill curves demonstrate a synergistic interaction of combinations of sub-MICs of oxacillin and vancomycin against clinical isolates MRSA (Domaracki et al., 1998). The combination of vancomycin and other β-lactam antibiotics had synergistic effect. The imipenem-vancomycin and cefazolin-vancomycin combinations reported strongly bactericidal against MRSA (Rochon-Edouard et al., 2000). Teicoplanin,

another glycopeptide antibiotic, was studied in combination with cefozopran and showed synergistic effect against 98% of MRSA strains (Toyokawa et al., 2003).

Rifampicin is a complex macrocyclic antibiotic that specifically inhibits chain initiation of bacterial DNA-dependent RNA polymerase by binding to the β-subunit of the enzyme (Wehrli et al., 1968). It is active against many pathogens including *S. aureus*, coagulase-negative staphylococci, and mycobacteria. Unfortunately, resistance occurs rapidly via 1-step target-site mutations in the B-subunit of RNA polymerase. Therefore, rifampicin must always be administered in combination with another antibiotic (Aubry-Damon et al., 1998). The combination between vancomycin and rifampicin are used to treat MRSA endocarditis (Faville Et al., 1978; Bayer and Lam, 1985) and septicemia (Gang et al., 1999).

Daptomycin is a lipopeptide antibiotic with bactericidal activity against MRSA. Like vancomycin, the combination with β-lactam antibiotics were studied and reported the significantly increased zone of inhibition when ampicillin/sulbactam and ticarcillin/clavulanate were combined with 1/2 MIC or 1/4 MIC of daptomycin against MRSA (Rand and Houck, 2004). Daptomycin was combined with aminoglycoside, such as gentamicin. Due to high bactericidal activity of daptomycin against MRSA, gentamicin was not showed any synergistic or additive effect to daptomycin (Tsuji and Rybak, 2005 ; DeRyke et al, 2006).

Combination between ampicillin and sulbactam or other β-lactam antibiotics and β-lactamase inhibitors, In vitro study reported the synergistic effect of ampicillin and sulbactam combination against MRSA (Kazmierczak et al., 1986). The other combinations, such as piperacillin/tazobactam also had synergistic effect (Palmer and Rybak, 1997).

CHAPTER III

MATERIALS & METHODS

1. Microorganisms

The bacterial isolates used throughout this study were 30 isolates of *mecA* positive methicillin resistant *S. aureus* (*mecA* positive MRSA). These bacteria were clinically isolated from the patients at Siriraj Hospital. The strains were kindly provided by Associated Professor Dr. Charnwit Tribuddharat from Faculty of Medicine, Siriraj Hospital. The standard strain used was *S. aureus* ATCC 29213.

2. Chemicals

Standard powder of ampicillin was kindly provided by Siam Bheasach, Thailand and standard powder of ampicillin/sulbactam was purchased from Pfizer, U.S.A. Working standard solutions were prepared immediately prior to use, as recommended by the manufacturers.

Ethanol 95% and nitrocefin disk were purchased from Government Pharmaceutical Organization, Bangkok and BBL chemicals (U.S.A), respectively.

3. Medicinal plant materials

The stem barks of *Hopea odorata* ROXB. were prepared from *H. odorata* ROXB. grew at Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand between June and July 2005. The plant materials were identified for the scientific name and botanical charateristics by Associated Professor Suratana Umnuaypol from Department of Pharmacogosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

3.1 Preparation of crude extract

3.1.1 The collected stem barks of the plant were dried in a hot air oven at 180°C for 2 days. Dried stem barks weight 1,000 grams were chopped to small pieces.

3.1.2 The small pieces of dried stem barks were macerated with 95% ethanol 5,000 ml, as a solvent for 2 days.

3.1.3 The solvent was then filtered using Whatman filter paper No.1 which separated the extracted from stem barks. The extract was concentrated using a rotatory evaporator at 40^0C.

3.1.4 From 1,000 grams of starting plant material, 249.47 grams of dry extracted were obtained. The extracted compound was brown colored and odorless.

3.1.5 The concentrated ethanol extract has been kept in the dessicator, the silica gel was used as a dessicant. The extract was dissolved in 95% ethanol before use.

3.2. Phytochemical screening

Chemical tests were carried out on the ethanol extract using standard procedures to identify the constituents as described by Sofawara (1993), Trease and Evans (1989) and Harborne (1973).

3.2.1 Test for tannins

0.5 g. of the dried powdered samples were boiled in 20 ml of water in a test tube and then filtered. The sample was divided into 3 portions, which the first portion was use as the control. A few drops of 1% gelatin solution were dropped into the second portion of the sample. A white turbid precipitate was observed indicating the presence of tannins. A few drops of 0.1% ferric chloride solution was added into the third portion. A brownish green or blue-black colouration was observed indicating the presence of tannins.

3.2.2 Test for flavonoids

0.5 g. of the dried powdered samples were dissolved in 50% ethanol and then filtered. The sample was divided into 2 portions, which the first portion was used as the control sample. One to two drops of concentrated HCl, and two or three pieces of magnesium ribbons were added to the second portion. A pink to red colouration in extract within 1-2 minutes was observed indicating the presence of flavonoids.

4. Detection of beta-lactamase

The nitrocefin disks which are impregnated with chromogenic cephalosporin or nitrocefin, were used. This compound exhibits a very rapid colour change from yellow to red as the amide bond in the beta-lactam ring is hydrolyzed by a beta-lactamase. When the bacterium produced this enzyme in significant quantities, the yellow-coloured disk turns red in the area where the isolate is smeared. Each disk is used to test one bacterial strain for the presence of beta-lactamase.

4.1 Nitrocefin-based test

The well-isolated colony of each 18 hours cultures of the clinical isolates and control strain of *S. aureus* were selected from Tryptic soy agar (TSA) plates. 20 µl of strile water was dropped to nitrocefin disks. The top of 1-2 well-isolated colonies were touched with a loop and tranfered on nitrocefin disks. The beta-lactamase activity was observed within 5 min.

4.2 Results and Interpretation

A positive result showed the color change of the disk from yellow to red on the area where the culture was applied. A negative result showed no colour change on the disk.

5. Antibiotic susceptibility test (NCCLS, 2004)

Paper disk susceptibility test was performed according to the Kirby-Bauer method by NCCLS (NCCLS, 2004). *S. aureus* ATCC 29213 was also included in this study as the control isolate. The susceptibility patterns of all 30 isolates against all the tested antimicrobial agents were determined

5.1 Preparation of media

5.1.1 Muller-Hinton agar (MHA) was prepared as directed to the manufacture's instructions.

5.1.2 Immediately after autoclaving, the media was allowed to cool in 45 °C water bath.

5.1.3 The freshly prepared and cooled medium was poured into glass, flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 25 ml for plates with a diameter of 100 mm.

5.1.4 The agar medium should be allowed to cool at room temperature and all prepared plates must be examined sterility by incubating at 37 °C for 24 hours.

5.1.5 Unless the plates were used within the same day, they were stored in a refrigerator (2 to 8 °C) and should be used within 7 days after preparation.

5.2 Alcoholic extract of. *H. odorata* ROXB. disk preparation

5.2.1 The extract was prepared at concentration of 1 mg/ml for 5 ml. The 20 µL of extract was dropped on the paper disc (Whatman no.1, 6 mm).

5.2.2 These paper disk were left in a sterile petri dishes until the solvent was completed vaporize at room temperature before use.

5.3 Inoculum Preparation

5.3.1 The well-isolated colony of each 18 hours from *S. aureus* clinical specimen and *S. aureus* ATCC 29213 were selected from Tryptic soy agar (TSA) plates and transferred to a tube containing 5 ml normal saline solution (NSS).

5.3.2 The suspension was adjusted to match the turbidity of the 0.5 McFarland standard solution. This result in a suspension containing approximately 1 to 2 x 10^8 CFU/ml.

5.4 Inoculation Test Plates

5.4.1 Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab.

5.4.2 The dried surface of an agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60 °C each time to ensure an even distribution of inoculum.

5.5 Application of Disks to Inoculated Agar Plates

5.5.1 The alcoholic extract of *H. odarata* ROXB. disks were applied to the surface of the medium with sterile forceps. Each disk must be pressed down to ensure complete contact with the agar surface. They must be distributed evenly so that they are no closer than 24 mm. from center to center. Because some of the drug diffuses almost instantaneously, a disk should not be relocated once it has come into contact with the agar surface. Instead, place a new disk in another location on the agar.

5.5.2 The plates were inverted and placed in ambient air incubator set to 37 °C within 15 minutes after the disks were applied in ambient air.

5.6 Reading Plates and Interpreting Results

5.6.1 The diameter of each zone of inhibition was measured with digital sliding venier caliper.

6. Agar dilution MIC determinations

Agar dilution method was performed according to NCCLS, 2004. All isolates and *S. aureus* ATCC 29213 were determined minimum inhibitory concentration (MIC) of ampicillin, ampicillin/sulbactam, and alcoholic extract of *H. odorata* ROXB.

6.1 Concentration preparation

6.1.1 The two-fold dilution of ampicillin solution (0.03-256 µg/ml) , ampicillin/sulbactam (0.03-256 µg/ml) and alcoholic extract of *H. odorata* ROXB. (0.25-16 mg/ml) were prepared. Thus the final volume in each plate consisted of 2.5 ml of each dilution of the antimicrobial agents or plant extract and 22.5 ml of MHA, so the stock solutions of each agent were prepared to be ten-fold greater than the desired final concentrations.

6.1.2 Mueller-Hinton agar (MHA) was prepared as directed to the manufacture's instructions.

6.1.3 Immediately after autoclaving, allow it to cool in a 55 °C water bath and then pipetted 2.5 ml of each dilution of the test agents into 22.5 ml of MHA.

6.1.4 The agar and antimicrobial agent solution were mixed thoroughly and then pour into plates.

6.1.5 The agar dilution plates were allowed to solidity at room temperature, and used immediately.

6.2 Inoculum preparation

6.2.1 The agar plates were marked for orientation of the inoculum spots.

6.2.2 A 1 µl of each inoculum was applied to the agar surface by the use of an inocula-replcating device. The final inoculum on the agar will then be approximately 10^4 CFU per spot.

6.2.3 A growth-control plate (no antimicrobial agent) was inoculated first and then, starting the lowest concentration, the plates containing the different concentrations were inoculated.

6.3 Incubating agar dilution plates

The incubated plates were allowed to stand at room temperature until the moisture in the inoculum spots have been absorbed into the agar until the spots were

dried, but no more than 30 minutes. The plates were inverted and incubated at 37 °C for 24 hours.

6.4 Determining agar dilution end points

6.4.1 The MICs were recorded as the lowest concentration of antimicrobial agent that completely inhibited the growth, disregarding a single colony or a faint haze caused by the inoculum.

6.4.2 The MICs were interpreted by referring to the NCCLS, 2004 and the organisms were reported as either susceptible, intermediate, or resistant to the agents that have tested (Table 3-1).

Table 3-1: MICs interpretive standards breakpoints (μg/ml) (NCCLS, 2004)

Drug	Minimum Inhibitory Concentration [MICs] (μg/ml)			
	S. aureus			*S. aureus*
	R[a]	I[b]	S[c]	ATCC 29213
ampicillin	≥0.5	-	≤0.25	0.5-2
Ampicillin/sulbactam	≥32/16	-	≤8/4	-

[a]Resistant, [b]intermediate, [c]Susceptible

7. Checkerboard synergy testing.

The checkerboard microdilution panel method served to determine the activity of alcoholic extract of *H. odorata* ROXB. in combination with ampicillin and ampicillin/sulbactam . The concentrations tested for ampicillin were 2, 4, 8, 16, 32, 64, 128 and 256 μg/ml, ampicillin/sulbactam were 1, 2, 4, 8, 16, 32, 64, 128 μg/ml, and for alcoholic extract of *H. odorata* ROXB. were 1.95, 3.9, 7.8, 15.6, 31.2, 62.5, 125, 250 μg/ml.

7.1 The twofold dilutions of drug or the extract were prepared volumetrically in the broth. The final volume of 200 μl in each well consisted of 50 μl of MHB , 50 μl of broth for drug, 50 μl of broth for extract and 50 μl of broth containing a suspension of the organism was obtained. Thus antimicrobial concentrations used in the initial (stock) solutions were prepared four-fold in greater than the desired final concentration. The concentrations tested for each antimicrobial and extract typically ranged from 5 dilutions below the MIC to twice the MIC or higher.

7.2 A series of antimicrobial and extract solutions containing four times the desired final concentrations were taken to produce the desired range of drug concentration by adding an aliquot of those solution to each well in the appropriate row or column (as shown in Figure 3-1).

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract

Drug

Figure 3-1: Checkerboard technique. In the checkerboard, serial dilution of drug and extract are performed using drug and extract proportional to MICs of the drug and extract being tested. (Modified from Eliopoulos and Moellering, 1996)

7.3 The interpretations of the antimicrobial combination interactions were done by reading the first clear well in each row of the panel with both agents. Based on this reading, the result of checkerboard study were interpreted by the pattern they form on the isobologram (Figure 3-2).

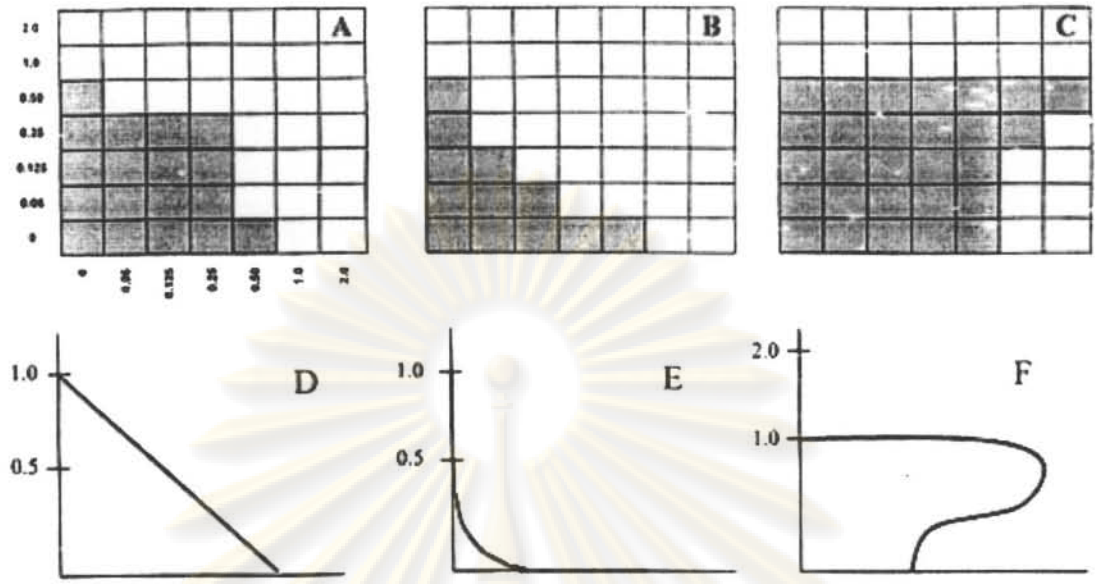


Figure 3-2: Assessment of antimicrobial combinations with the checkerboard method. A, B, and C. Results of testing combinations of drug and extract. Shading, visible growth. Concentrations are expresses as multiples of MIC. Isobolograms (plotted on an arithmetic scale) that represent the results of checkerboards shown in D,E, and F, respectively. A and D. Additive effect. B and E. Synergism. C and F. Antagonism. (Modified from Eliopoulos and Moellering, 1996).

7.3.1 To evaluate the effect of the combinations, the fractional inhibitory concentration were calculated for each antimicrobial alone and in combination. The following formular were used to calculate the FIC.

$$\text{FIC of antimicrobials} = \frac{\text{MIC of antimicrobials in combination}}{\text{MIC of antimicrobials alone}}$$

$$\text{FIC of extract} = \frac{\text{MIC extract in combination}}{\text{MIC of extract alone}}$$

$$\text{FIC index } (\Sigma\text{FIC}) = \text{FIC of antimicrobials} + \text{FIC of extract}$$

7.3.2 FIC index results for each combination were defined as:

7.3.2.1 synergy, if the decrease in the MIC of each agent was ≥4-fold (FIC index ≤ 0.5)

7.3.2.2 partial synergy, if the decrease in the MIC of 1 agent was ≥4-fold and the decrease in the MIC of the other agent was 2-fold (FIC index, >0.5 and <0.1).

7.3.2.3 additive, if the decrease in the MIC of both agent was 2-fold (FIC index = 1).

7.3.2.4 indifference, if the interaction did not meet the above criteria and were not antagonistic (FIC index, >1 and <4).

7.3.2.5 antagonism, if an increase in the MIC of both agents was ≥4-fold (FIC index ≥ 4).

The smallest FIC value was used to establish the antimicrobial combination interaction for each specific strain, except for antagonism, which was preferably reported. Results were expressed as percentage of isolates with synergism, additive, indifference, and antagonism.

8. Time kill assays

The antibacterial activity of the combination was performed according to the time kill method by Elipoulos and Moellering, 1996. Drug concentration used for the time kill assays were based on criteria (i) concentration likely to produce synergy. partial synergy and additive as seen in checkerboard testing (ii) concentration that were no more than of each drug.

8.1 Alcoholic extract of *H. odorata* ROXB. and antimicrobials concentrations(ampicillin, ampicillin/sulbactam) used in initial (stock) solutions were prepared four fold, two fold greater than desired final concentration, respectively.

8.2 A 5 ml of each alcoholic extract of *H. odorata* ROXB. and antimicrobials were pipetted into Mueller Hinton broth (MHB) for prepared working media adding the standardized inoculum (final volume of working media = 5 ml). As the result, there had been 7 groups of control (no antimicrobial agents), extract 1/2 MIC alone, extract 1/4 MIC alone, antimicrobials 1/2 MIC alone, antimicrobials 1/4 MIC or 1/8 MIC alone, extract 1/2 MIC combined with antimicrobials 1/4 MIC or 1/8 MIC and extract 1/4 MIC combined with antimicrobials 1/2 MIC.

8.3 Inoculum which was adjusted to match the turbidity of the 0.5 McFarland standard solution, contained approximately 1 to 2 x 10^8 CFU/ml. was then diluted ten fold to make 1 to 2 x 10^7 CFU/ml. of the bacterial inoculum.

8.4 A 5 ml of inoculum was pipetted into the working media and incubated at 37 °C in shaking waterbath.

8.5 The samples were collected for culture at the time 0, 2, 4, 6, 8, 10 and 24 hours after the microorganism was exposed to in each group of the antimicrobials including the control group. A 0.5 ml of the collected sample was diluted ten fold in NSS and 20 µl of each dilution was dropped on TSA plates which were then incubated at 37 °C for 16-18 hours.

8.6 The quantity of survival bacteria in each group was calculated to obtain the killing curves data. The quantity of survival bacteria in each group was calculated to obtain the killing curves data. Killing curve were constructed by Microsoft Excel 2002 at each time interval. The $\log_{10}$ change of the viable cell counts compared to the starting inoculum was determined.

8.6.1 The results were analyzed by determining the number of strains which yield changes in the $\log_{10}$ number of CFU/ml of -1,-2, and -3 at 2, 4, 6, 8, 10 and 24 hours compared to the counts at 0 hours. A given concentration of antimicrobial alone or in combination was considered bactericidal of it reduced the original inoculum size by ≥ 3 $\log_{10}$ CFU/ml ($\geq$ 99.9% killing) at each of the time periods or bacteriostatic if the inoculum size was reduced by 0-3 $\log_{10}$ CFU/ml. The regrowth was defined as an increase of $\geq$ 2 log CFU/ml after $\geq$6 hours. (Pankuch et al., 1994; Amsterdam, 1996;).

8.6.2 The quantitative evaluation of antimicrobial effect was calculated as in the published article (Firsov et al., 1997).

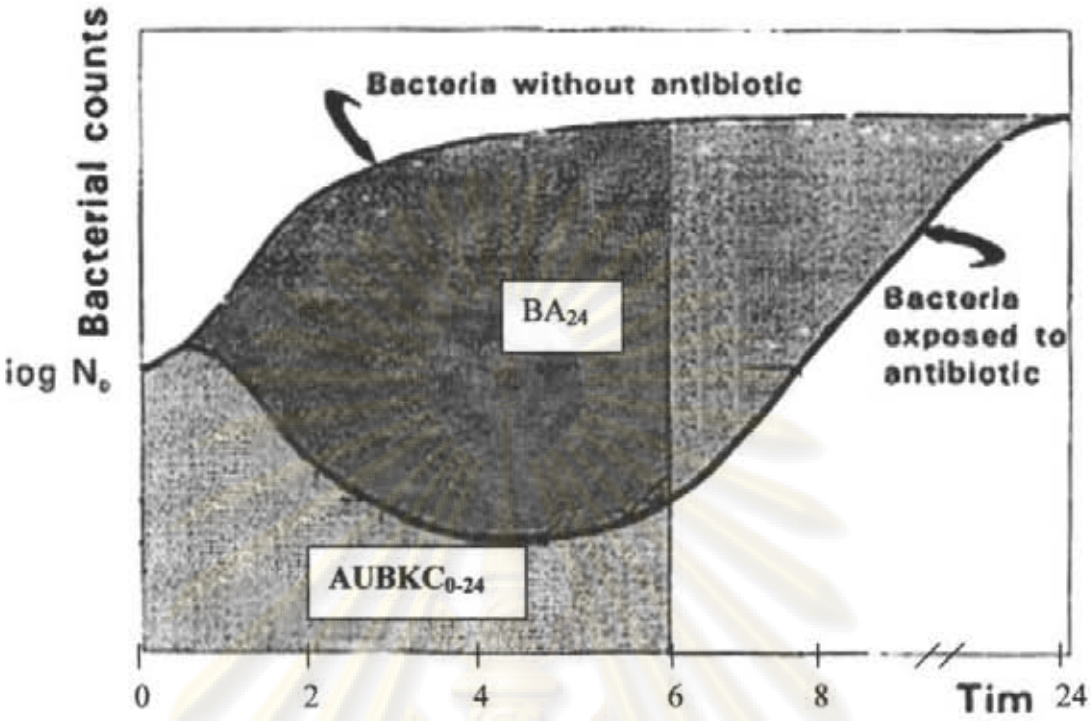


Figure 3-3: Parameters for quantifying bacterial killing and regrowth curve and the antimicrobial effect. (Modified from Firsov et al., 1997).

The following parameters were calculated by various methodologies as follow:

$AUBKC_{0\text{-}24}$ = Area under the bacterial killing and regrowth curves that were calculated by the trapezoidal rule for 24 hours.

Bacteriolytic area for 24 hours (BA_{24}) = the area between control growth curve and the bacterial killing and regrowth curves ($AUBKC_{0\text{-}24}$ of the control growth curve substracted by $AUBKC_{0\text{-}24}$ of the bacterial killing and regrowth curves).

Statistic analysis

Student's t-test was used to compared the Log change of viable cell counts, $AUBKC_{0\text{-}24}$ and BA_{24}, which expressed their mean value (± SEM) values. Any value of P below 0.05 was considered as significant.

CHAPTER IV

RESULT

1. Phytochemical screening test

This study was carried out on the alcoholic extract of *H. odorata* ROXB in order to reveal the presence of the biologically active constituents. The phytochemical characteritics of the *H. odorata* ROXB were summarized in Table 4-1. Tannin was present in this plants but alkaloids and flavonoid were not found in this alcohol extract.

Table 4-1: Qualitative analysis of the phytochemicals of the medicinal plant.

plant	tannin	flavonoid
H. odorata ROXB.	+	-

+ = Presence of constituent, - = Absence of constituent

2. Detection of beta-lactamase activity (Raw data were shown in Table A-1 in Appendices.)

From nitrocefin-base test, 29 out of 30 isolates of MRSA presenced positive reaction indicating that 29 isolates were produced except No. 643.

3. Susceptibility test

3.1 Disk diffusion method

Consequently, in order to detect a potential antimicrobial activity in alcoholic extract of *H. odorata* ROXB. against MRSA the disk diffusion method was performed. The diameter of the inhibition zones by the alcoholic extract of *H. odorata* ROXB. at the concentration of 20 µg /disk. ranged from 6.42 - 10.08 mm. All strains expect one isolate (no.200) were inhibited by the alcoholic extract of *H. odorata* ROXB. (Table A-1 in Appendices).

3.2 Agar dilution method

The range of MICs of ampicillin, ampicillin/sulbactam and oxacillin, the MIC_{50} and MIC_{90} of all tested drugs and the percentage of susceptible isolate to ampicillin, ampicillin/sulbactam and oxacillin against the 30 isolates were shown in Table 4-2. Ampicillin had no activity against all strains tested. MICs of ampicillin ranged from 8-128 μg/ml which were the high level of resistance (susceptibility breakpoint ≤0.25 μg/ml). The MIC_{50}, MIC_{90} of ampicillin were 64, 128 μg/ml, respectively. One isolate strain (No.1028) was susceptible to ampicillin/sulbactam (susceptibility breakpoint ≤8 μg/ml). The MIC_{50}, MIC_{90} of ampicillin/sulbactam were 32 μg/ml. MICs of oxacillin ranged from 256->256 μg/ml. The MIC_{50}, MIC_{90} of oxacillin were >256 μg/ml. The MICs of *H. odorata* Roxb ranged from 62.5-125 μg/ml which were the high level of MICs. The MIC_{50}, MIC_{90} of *H. odorata* Roxb were 125 μg/ml. (Raw data of susceptibility testing by agar dilution method were shown in Table A-2 in Appendices.)

Table 4-2: *In vitro* activity of ampillin ampicillin/sulbactam and oxacillin against 30 isolate of *S.aureus* as tested by agar dilution method.

	MICs (μg/ml)		
	Range	MIC_{50}	MIC_{90}
Alcoholic extract of *H.odorata*	62.5-125	125	125
ampicillin	8-128	64	128
ampi/sul	8-32/ND	32/ND	32/ND
oxacillin	256 - >256	>256	>256

ND = not determined

4. Synergy test (Raw Data of checkerboard were shown in Appendices: Figure A-1 to A-30 for ampicillin plus extract and A-31 to A-60 for ampicillin/sulbactam plus extract).

Checkerboard method was used to assess the MIC and the synergistic activity of two antimicrobial agent combinations including alcoholic extract of *H. odorata* ROXB. plus ampicillin against 30 isolates of MRSA (Table 4-3); alcoholic extract of

H. odorata ROXB. plus ampicillin/sulbactam against 30 isolates of MRSA (Table 4-4). The MICs of both ampicillin and ampicillin/sulbactam in combination with 1/2 MIC of the alcohol extract were decrease 2-8 fold against 30 isolates of MRSA (Table 4-5)

Table 4-3: MICs of ampicillin , *H. odorata* ROXB and the combination of alcoholic extract *H. odorata* ROXB plus ampicillin against 30 isolates of MRSA by checkerboard method. (A* = ampicillin; H* = *H. odorata* ROXB)

Strain	β-lactamase	*mecA*	MIC alone (μg/ml)		MIC A* combined with 1/2H	FIC index	interpretation	MIC A* combined with 1/4H	FIC index	interpretation
			H*	A*						
3	+	+	31.2	32	8	0.75	P	32	1.25	I
9	+	+	31.2	64	32	1	A	64	1.25	I
17	+	+	31.2	64	8	0.63	P	32	0.75	P
19	+	+	31.2	64	16	0.75	P	64	1.25	I
20	+	+	31.2	32	8	0.75	P	16	0.75	P
23	+	+	31.2	32	8	0.75	P	16	0.75	P
31	+	+	31.2	32	16	1	A	32	1.25	I
32	+	+	31.2	64	16	0.63	P	32	0.75	P
34	+	+	31.2	32	16	1	A	32	1.25	I
38	+	+	31.2	64	16	0.75	P	64	1.25	I
94	+	+	31.2	32	8	0.75	P	32	1.25	I
102	+	+	31.2	64	16	0.75	P	64	1.25	I
107	+	+	31.2	32	4	0.63	P	8	0.50	S
152	+	+	31.2	64	8	0.63	P	32	0.75	P
200	+	+	31.2	64	16	0.75	P	64	1.25	I
216	+	+	31.2	64	16	0.75	P	64	1.25	I
234	+	+	31.2	64	8	0.63	P	32	0.75	P
240	+	+	31.2	64	32	1	A	64	1.25	I
241	+	+	31.2	64	16	075	P	64	1.25	I
266	+	+	31.2	64	16	0.75	P	32	0.75	P
268	+	+	31.2	64	16	0.75	P	32	0.75	P
269	+	+	31.2	32	8	0.75	P	32	1.25	I
279	+	+	31.2	32	16	1	A	32	1.25	I
384	+	+	31.2	64	16	0.75	P	32	0.75	P
466	+	+	31.2	64	32	0.56	P	32	0.75	P
643	-	+	31.2	32	16	1	A	16	0.75	P
777	+	+	31.2	64	32	0.63	P	32	0.75	P
786	+	+	31.2	32	16	1	A	32	1.25	I
F9	+	+	31.2	64	32	1	A	64	1.25	I
1028	+	+	31.2	8	2	0.75	P	2	0.50	P
Range				8-64	2-32	0.56-1		2-64	0.50-1.25	

Table 4-4: MICs of ampicillin/sulbactam , *H. odorata* ROXB and the combination of alcoholic extract *H. odorata* ROXB plus ampicillin/sulbactam against 30 isolates of MRSA by checkerboard method. (A/S* = ampicillin/sulbactam; H* = *H. odorata* ROXB)

Strain	β-lactamase	*mecA*	MIC alone (μg/ml)		MIC A/S* combined with 1/2H	FIC index	interpretation	MIC A/S* combined with 1/4H	FIC index	interpretation
			H*	A/S*						
3	+	+	31.2	32	8	0.75	P	16	0.75	P
9	+	+	31.2	16	4	0.75	P	16	1.25	I
17	+	+	31.2	16	8	1	A	16	1.25	I
19	+	+	31.2	16	2	0.63	P	16	1.25	I
20	+	+	31.2	16	2	0.63	P	8	0.75	P
23	+	+	31.2	16	2	0.63	P	8	0.75	P
31	+	+	31.2	16	2	0.63	P	8	0.75	P
32	+	+	31.2	16	2	0.63	P	8	0.75	P
34	+	+	31.2	16	8	1	A	16	1.25	I
38	+	+	31.2	16	8	1	A	16	1.25	I
94	+	+	31.2	16	4	0.75	P	8	0.75	P
102	+	+	31.2	32	8	0.75	P	16	0.75	P
107	+	+	31.2	16	8	1	A	16	1.25	I
152	+	+	31.2	16	4	0.75	P	16	1.25	I
200	+	+	31.2	16	8	1	A	16	1.25	I
216	+	+	31.2	64	4	0.56	P	16	1.25	I
234	+	+	31.2	16	8	1	A	16	1.25	I
240	+	+	31.2	16	8	1	A	16	1.25	I
241	+	+	31.2	16	8	1	A	16	1.25	I
266	+	+	31.2	16	8	1	A	16	1.25	I
268	+	+	31.2	16	8	1	A	16	1.25	I
269	+	+	31.2	16	4	0.75	P	16	1.25	I
279	+	+	31.2	32	8	0.75	P	16	0.75	P
384	+	+	31.2	16	8	1	A	16	1.25	I
466	+	+	31.2	16	8	1	A	16	1.25	I
643	-	+	31.2	16	4	0.75	P	16	1.25	I
777	+	+	31.2	32	8	0.75	P	16	0.75	P
786	+	+	31.2	32	8	1	A	16	0.75	P
F9	+	+	31.2	32	8	0.75	P	32	1.25	I
1028	+	+	31.2	8	2	0.75	P	4	0.75	P
Range				8-64	2-8	0.56-1		4-32	0.75-1.25	

Table 4-5: Number of isolate were decrease by the combination of alcoholic extract *H. odorata* ROXB plus ampicillin and ampicillin/sulbactam against 30 isolates.

MIC of A or A/S in combination were decrease	Number(%) of isolate were decreased by			
	A+¼H	A+½H	A/S+¼H	A/S+½H
2 fold	12(40)	12(40)	11(36.67)	12(40)
4 fold	2(6)	11(36.67)	1(3)	12(40)
8 fold	ND	7	ND	5(16.67)
16 fold	ND	ND	ND	1(3)

A = ampicillin, H = *H. odorata* ROXB, A/S = ampicillin/sulbactam
ND = not determine

The MICs of *H. odorata* ROXB were 31.2 µg/ml against the 30 isolates of MRSA. The combination of ampicillin plus 1/2 MIC of the alcoholic extract of *H. odorata* ROXB it was shown that the MIC of ampicillin in the combination was decreased 2-8 folds against all 30 isolates of MRSA (100%) as compared to the MIC of ampicillin alone. FIC index of ampicillin plus 1/2 MIC of the alcoholic extract of *H. odorata* ROXB ranged from 0.56-1.When the concentration of the extract was decrease to 1/4 MIC, it was shown that the MIC was decreased 2-4 folds against 14 isolates (46.67%) as compared to the MIC of ampicillin alone. The MIC of the combination ampicillin plus the alcoholic extract *H. odorata* ROXB was not decreased below susceptibility breakpoint (≤ 0.25 µg/ml) but lower than the MIC of ampicillin alone. So all of 30 MRSA isolates still resisted to ampicillin. FIC index of ampicillin plus 1/4 MIC of the alcoholic extract of *H. odorata* ROXB ranged from 0.50-1.25 (Table 4-3 and Table 4-5).

When ampicillin/sulbactam was conbined to 1/2 MIC of the alcoholic extract *H. odorata* ROXB, it was shown that the MIC of ampicillin/sulbactam in this combination was decreased 2-16 folds against all 30 isolates of MRSA (100%) as compared to the MIC of ampicillin/sulbactam alone. Additionally, the MIC of ampicillin/sulbactam in this combination were lower than the susceptibility breakpoint (≤ 8 µg/ml), against all 30 isolates (100%) which meant that all MRSA strains were not resist to ampicillin/sulbactam when combined with the alcoholic extract *H. odorata* ROXB. FIC index of ampicillin/sulbactam plus 1/2 MIC of the alcoholic extract of *H. odorata* ROXB ranged from 0.63-1. When of ampicillin/sulbactam was combined to the ¼ MIC of the alcoholic extract *H. odorata* ROXB, it was shown that the MIC was decreased 2-4 folds against 12 isolates (40%) as compared to the MIC of ampicillin alone. The MIC were lower than the

susceptibility breakpoint (≤ 8 μg/ml) against 6 strains (20%). FIC index of ampicillin plus 1/4 MIC of the alcoholic extract of *H. odorata* ROXB ranged from 0.75-1.25 (Table 4-4 and Table 4-5).

Table 4-6: Effect of the combination of alcoholic extract *H. odorata* ROXB. plus ampicillin against 30 isolates of MRSA by checkerboard method.

Effect	Combination [number (%) of isolates]	
	1/2 MIC alcoholic extract *H. odorata* ROXB. plus ampicillin	1/4 MIC alcoholic extract of *H. odorata* ROXB. plus ampicillin
Synergism	-	2(6.67)
Partial synergy	22(73.33)	12(40)
Additive	8(26.67)	-
Indifference	-	16 (53.33)

The combination of ampicillin plus 1/2 MIC alcoholic extract *H. odorata* ROXB showed the partial synergistic effect against 22 isolates (73.33%) and additive effect in 8 isolates (26.67%). The combination of ampicillin plus 1/4 MIC alcoholic extract *H. odorata* ROXB showed the synergistic effect in 2 isolates (6.67%), partial synergism effect in 12 isolates (40%) and indifference effect in 16 isolates (53.33%).

Table 4-7: Effect of the combination of alcoholic extract of *H. odorata* ROXB. plus ampicillin/sulbactam against 30 isolate of MRSA by checkerboard method.

Effect	Combination [number (%) of isolates]	
	1/2 MIC alcoholic extract *H. odorata* ROXB. plus ampi/sul	1/4 MIC alcoholic extract of *H. odorata* ROXB. plus ampi/sul
Synergism	-	-
Partial synergy	18(60)	12(40)
Additive	12(40)	-
Indifference	-	18(60)

The combination of ampicillin/sulbactam plus 1/2 MIC alcoholic extract *H. odorata* ROXB showed the partial synergism effect against 18 isolates (60%) and additive effect in 12 isolates (40%). The combination of ampicillin plus 1/4 MIC alcoholic extract *H. odorata* ROXB showed the partial synergism effect in 12 isolates (40%) and indifference effect in 18isolates (60%). As summarized in table 4-3 and table 4-4 FIC index of ampicillin and ampicillin/sulbactam were from 0.5-1.25 in combination with 1/4 MIC (7.8 μg/ml) or 1/2 MIC (15.6 μg/ml) of *H. odorata* ROXB.

against 30 isolates of MRSA no antagonist was observed between ampicillin or ampicillin/sulbactam.

Table 4-8: MICs of ampicillin , ampicillin/sulbactam , *H. odorata* ROXB and the combination of alcoholic extract *H. odorata* ROXB plus ampicillin and ampicillin/sulbactam were defined as partial synergism effect against 18 isolates of MRSA.

Strain no.	MIC (µg/ml) ampicillin			MIC (µg/ml) ampicillin/sul		
	alone	Plus ½ H	Plus ¼ H	alone	Plus ½ H	Plus ¼ H
3	ND	ND	ND	32	8	16
17	64	8	32	ND	ND	ND
20	32	8	16	16	2	8
23	32	8	16	16	2	8
31	ND	ND	ND	16	2	8
32	64	16	32	16	2	8
94	ND	ND	ND	16	4	8
102	ND	ND	ND	32	8	16
107	32	4	16	ND	ND	ND
234	64	8	32	ND	ND	ND
266	64	16	32	ND	ND	ND
268	64	16	32	ND	ND	ND
279	ND	ND	ND	32	8	16
384	64	16	32	ND	ND	ND
466	64	4	32	ND	ND	ND
777	64	8	32	32	4	16
786	ND	ND	ND	32	8	16
1028	ND	ND	ND	8	2	4

ND = the combination of alcoholic extract *H. odorata* ROXB plus ampicillin and ampicillin/sulbactam were not defined as partial synergism effect, H = *H. odorata* ROXB.

When ampicillin plus 1/2 MIC of the alcoholic extract *H. odorata* ROXB, it was shown that the MIC of ampicillin in this combination were decreased 8 fold against 5 isolates of MRSA (45.46%) no. 17, 107, 234, 466, and 777 as compared to the MIC of ampicillin alone (Figure 4-1) and were decreased 4 fold against 6 isolates of MRSA (54.54%) no.20, 23, 32, 266, 268, and 384 compared to the MIC of ampicillin alone (Figure 4-2).

When ampicillin/sulbactam plus 1/2 MIC of the alcoholic extract *H. odorata* ROXB, it was shown that the MIC of ampicillin in this combination were decreased 8 fold against 4 isolates of MRSA (36.36%) no. 20, 23, 31, and 32 as compared to the MIC of ampicillin/sulbactam alone (Figure 4-3) and were decreased 4 fold against 7

isolates of MRSA (63.63%) no.3, 94, 102, 279, 777, 786, and 1028 as compared to the MIC of ampicillin/sulbactam alone (Figure 4-4).

When ampicillin or ampicillin/sulbactam was combined to 1/4 MIC of the alcoholic extract *H. odorata* ROXB., it was shown that the MIC of ampicillin and ampicillin/sulbactam in this combination were decreased 2 folds against 11 isolates of MRSA (36.67%) (Raw data were shown in appendices table A- 3 and A-4).

The synergistic interactions between alcoholic extract of *H. odorata* ROXB. plus ampicillin or plus ampicillin/sulbactam in this study were not only assessed from the MIC value but were also evaluated from the graph shape plotted on the isobologram and the fractional inhibitory concentration (FIC) index that were modified from checkerboard result as described in chapter III (method section). The graph shape of the alcoholic extract of *H.odorata* ROXB. plus ampicillin in 11 isolates of MRSA(no. 17, 20, 23, 32, 107, 234, 266, 268, 384, 466, and 777) were in the concave shape and were defined as partial synergism effect (shown in Figure A-61 to Figure A-66 in appendices) The graph shape of the alcoholic extract of *H.odorata* ROXB.plus ampicillin/sulbactam in 11 isolates of MRSA (no. 3, 20, 23, 31, 32, 94, 102, 279, 777, 786, and 1028) were in the concave shape and were defined as partial synergism effect. (shown in Figure A-67 to Figure A-70 in appendices.)

5. Time kill studies (Raw data were shown in Appendices Table A-3 to A-4)

The antibacterial activity of the combination between alcoholic extract of *H. odorata* ROXB. and ampicillin against 11 isolates of MRSA (no.17, 20, 23, 32, 107, 234, 266, 268, 384, 466, and 777) and the combination between alcoholic extract of *H. odorata* ROXB. and ampicillin/sulbactam against 11 isolates of MRSA (no.3, 20, 23, 31, 32, 94, 102, 279, 777, 786, and 1028) were tested by time kill method. The mean $\log_{10}$ decrease of viable cell counts and bacteriolytic area for 24 hours (BA_{24}) by the combination of extract of *H. odorata* ROXB. plus ampicillin were shown in Figure 4-1 to Figure 4-2, Table 4-9 and Table 4-10 and effect of the combination between extract of *H. odorata* ROXB. and ampicillin/sulbactam were shown in Figure 4-3 to Figure 4-4 and Table 4-11 and Table 4-12. All combination of alcoholic extract (*H. odorata* ROXB) plus ampicillin or plus ampicillin/sulbactam were shown bacteriostatic activity against 18 isolates of MRSA.

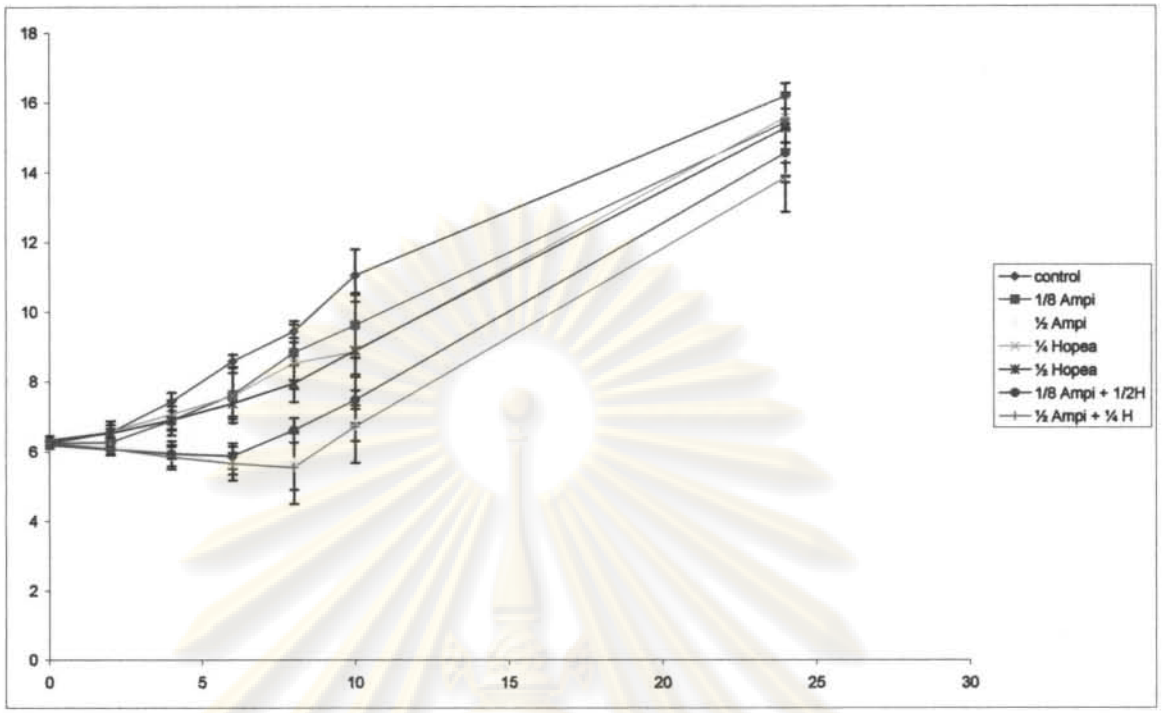


Figure 4-1: Time kill curves showing the bacteriostatic activity of the combination of 1/2 MIC alcoholic extract of *H. odorata* ROXB plus 1/8 MIC ampicillin against 5 isolates of MRSA (no. 17, 107, 234, 466, and 777).

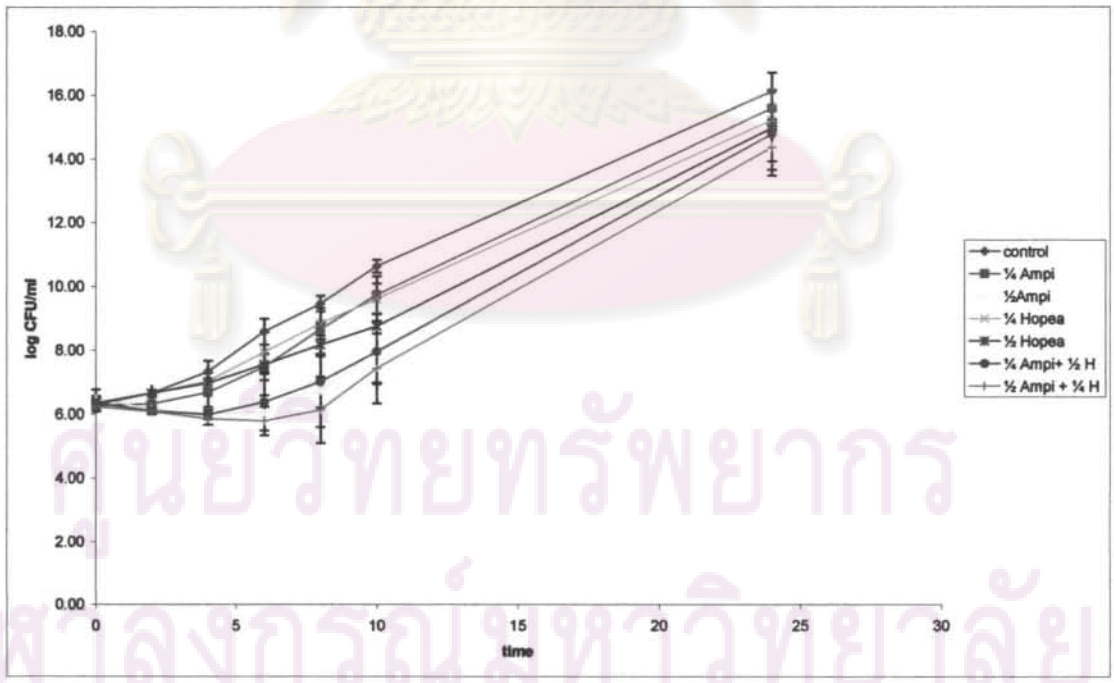


Figure 4-2: Time kill curves showing the bacteriostatic activity of the combination of 1/2 MIC alcoholic extract of *H. odorata* ROXB plus 1/4 MIC ampicillin against 6 isolates of MRSA (no. 20, 23, 32, 266, 268, and 384).

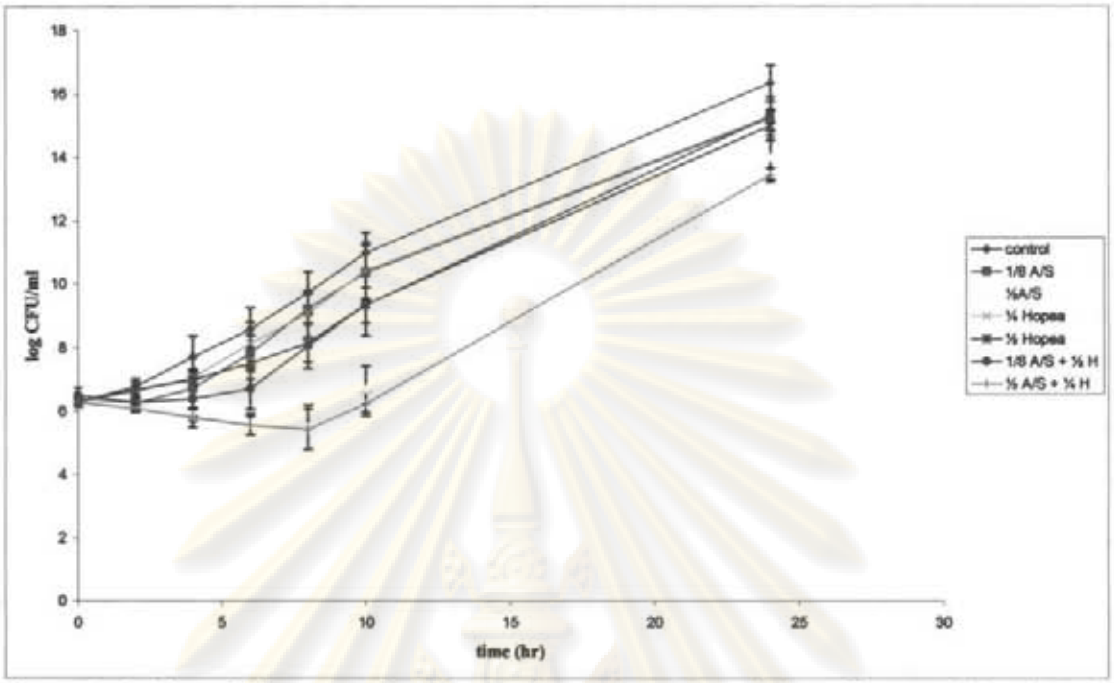


Figure 4-3: Time kill curves showing the bacteriostatic activity of the combination of 1/2 MIC alcoholic extract of *H. odorata* ROXB plus 1/8 MIC ampicillin/sulbactam against 4 isolates of MRSA (no.20, 23, 31, and 32).

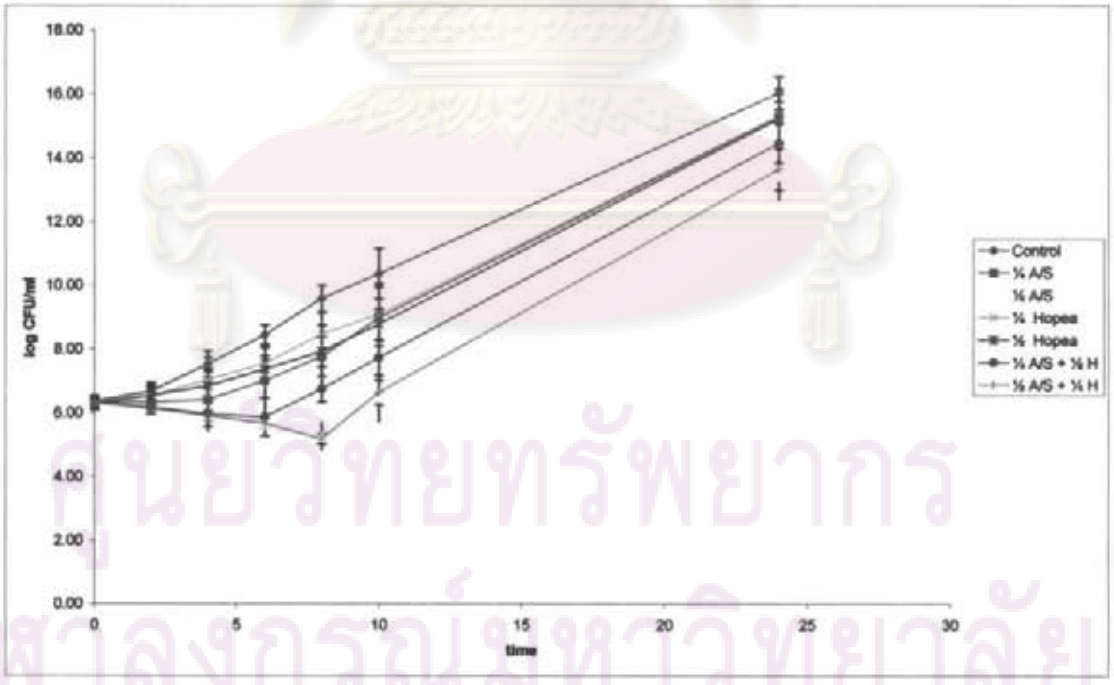


Figure 4-4: Time kill curves showing the bacteriostatic activity of the combination of 1/2 MIC alcoholic extract of *H. odorata* ROXB plus 1/4 MIC ampicillin/sulbactam against isolates of MRSA (no.3, 94, 102, 279, 777, 786, and 1028).

Table 4-9: Mean log change viable counts at various time interval, $AUBKC_{0-24}$ and BA_{24} in 5 isolates of MRSA no.17, 107, 234, 466, and 777. (1/8 MIC ampicillin plus 1/2 MIC alcoholic extract of *H. odorata* ROXB.)

condition	Mean(±SD) log change viable cell counts						Mean(±SEM) $AUBKC_{0-24}$	Mean(±SEM) BA_{24}
	Δ2	Δ4	Δ6	Δ8	Δ10	Δ24		
control	0.30±0.11	0.89±0.15	1.15±0.15	0.85±0.20	1.61±0.42	5.13±0.33	277.93±3.93	-
A1/8	0.02±0.04	0.63±0.13	0.74±0.22	0.94±0.43	0.79±0.14	5.80±0.25	250.31±7.28	27.45±10.84[a]
A1/2	-0.08±0.03	-0.17±0.04	-0.32±0.07	0.06±0.34	1.10±0.21	7.56±0.37	209.01±4.90	68.92±8.47[c]
H1/4	0.29±0.14	0.48±0.19	0.53±0.15	0.94±0.23	0.32±0.54	6.71±0.59	246.07±7.97	31.85±11.36
H1/2	0.20±0.11	0.63±0.09	0.57±0.12	0.58±0.15	0.94±0.15	6.37±0.39	241.87±5.60	35.88±9.22
A1/8+H1/2	-0.12±0.04	-0.12±0.10	-0.12±0.25	0.74±0.11	0.86±0.25	7.07±0.21	216.80±4.62	64.04±9.29[b]
A1/2+ H1/4	-0.16±0.04	-0.23±0.10	-0.19±0.13	-0.12±0.26	1.17±0.16	7.13±0.16	203.09±8.32	74.84±11.19[c]

Alphapet difference was significant at 0.05 level. Δ = Mean log change viable cell counts at 2, 4 , 6 , 8, and 24 hours, respectively.
$AUBKC_{0-24}$ = Area under bacterial killing and regrowth curves for 24 hours. BA_{24} = Bacterolytic area for 24 hours

Table 4-10: Mean log change viable counts at various time interval, $AUBKC_{0-24}$ and BA_{24} in 6 isolates of MRSA no.20, 23, 32, 266, 268, and 384. (1/4 MIC ampicillin plus 1/2 MIC alcoholic extract of *H. odorata* ROXB.)

condition	Mean(±SD) log change viable cell counts						Mean(±SEM) $AUBKC_{0-24}$	Mean(±SEM) BA_{24}
	Δ2	Δ4	Δ6	Δ8	Δ10	Δ24		
control	0.43±0.08	0.68±0.15	1.25±0.20	0.88±0.17	1.17±0.17	5.49±0.28	268.27±1.82	-
A1/4	0.08±0.11	0.40±0.14	0.80±0.08	1.18±0.13	1.04±0.16	5.72±0.32	249.84±4.26	18.43±4.40[a]
A1/2	-0.36±0.11	-0.15±0.05	0.03±0.20	0.70±0.30	1.18±0.12	6.47±0.16	220.83±6.58	47.43±6.95[c]
H1/4	0.42±0.05	0.38±0.08	0.91±0.23	0.90±0.15	0.77±0.24	5.45±0.11	249.55±3.10	18.72±2.95
H1/2	0.41±0.08	0.30±0.08	0.58±0.11	0.61±0.11	0.59±0.13	6.21±0.23	239.86±3.48	28.39±3.67
A1/4+ H1/2	-0.17±0.04	-0.18±0.03	0.39±0.32	0.64±0.14	0.94±0.13	6.82±0.37	224.50±6.39	43.77±6.54[b]
A1/2+ H1/4	-0.18±0.04	-0.23±0.05	-0.07±0.15	0.42±0.28	1.30±0.13	6.92±0.23	214.30±7.21	53.97±6.50[c]

Alphapet difference was significant at 0.05 level. Δ = Mean log change viable cell counts at 2, 4 , 6 , 8 and 24 hours, respectively.
$AUBKC_{0-24}$ = Area under bacterial killing and regrowth curves for 24 hours. BA_{24} = Bacterolytic area for 24 hours

Table 4-11: Mean log change viable counts at various time interval, $AUBKC_{0-24}$ and BA_{24} in 4 isolates of MRSA no.20, 23, 31, and 32. (1/8 MIC ampicillin/sulbactam plus 1/2 MIC alcoholic extract of *H. odorata* ROXB.)

condition	Mean(±SD) log change viable cell counts						Mean(±SEM) $AUBKC_{0-24}$	Mean(±SEM) BA_{24}
	Δ2	Δ4	Δ6	Δ8	Δ10	Δ24		
control	0.49±0.15	0.94±0.27	0.87±0.11	1.14±0.03	1.26±0.13	5.37±0.40	274.23±5.59	-
A/S1/8	-0.13±0.12	0.47±0.29	1.28±0.13	1.40±0.11	1.13±0.26	4.88±0.38	259.59±6.59	17.64±1.60[a]
A/S1/2	-0.14±0.05	-0.35±0.13	-0.27±0.09	0.41±0.22	1.19±0.13	7.30±0.43	204.35±5.10	69.86±2.56[c]
H1/4	0.31±0.11	0.45±0.13	1.07±0.34	0.98±0.16	1.36±0.36	4.87±0.47	258.57±4.32	15.67±1.31
H1/2	-0.22±0.17	0.12±0.17	0.31±0.11	1.35±0.12	1.27±0.48	5.96±0.41	236.47±5.41	29.52±4.35
A/S1/8+ H1/2	-0.19±0.10	-0.29±0.26	-0.25±0.36	-0.13±0.18	1.06±0.29	7.20±0.25	195.70±7.99	37.76±9.37[b]
A/S1/2+ H1/4	0.39±0.03	0.31±0.12	0.52±0.04	0.64±0.23	1.18±0.13	5.66±0.25	244.71±2.10	78.70±5.50[c]

Alphapet difference was significant at 0.05 level. Δ = Mean log change viable cell counts at 2, 4 , 6 , 8 and 24 hours, respectively.
$AUBKC_{0-24}$ = Area under bacterial killing and regrowth curves for 24 hours. BA_{24} = Bacterolytic area for 24 hours

Table 4-12: Mean log change viable counts at various time interval, $AUBKC_{0-24}$ and BA_{24} in 4 isolates of MRSA no.3, 94, 102, 279, 777, 786, and 1028. (1/8 MIC ampicillin/sulbactam plus 1/2 MIC alcoholic extract of *H. odorata* ROXB.)

condition	Mean(±SD) log change viable cell counts						Mean(±SEM) $AUBKC_{0-24}$	Mean(±SEM) BA_{24}
	Δ2	Δ4	Δ6	Δ8	Δ10	Δ24		
control	0.31±0.15	0.85±0.17	0.91±0.07	1.14±0.11	0.78±0.22	5.67±0.43	264.47±1.66	-
A/S1/4	-0.02±0.07	-0.03±0.10	0.60±0.16	0.75±0.19	1.21±0.19	6.31±0.43	238.74±4.93	25.75±4.80[a]
A/S1/2	-0.16±0.02	-0.25±0.06	-0.19±0.10	0.11±0.21	1.19±0.21	6.93±0.27	197.58±3.99	66.89±2.91[c]
H1/4	0.24±0.08	0.50±0.10	0.51±0.15	0.73±0.25	0.62±0.25	6.19±0.49	244.80±4.76	19.67±3.73
H1/2	0.24±0.06	0.30±0.06	0.38±0.16	0.55±0.12	0.83±0.12	6.44±0.27	240.17±3.66	24.31±3.67
A/S1/4+ H1/2	-0.10±0.02	-0.22±0.05	-0.10±0.18	0.87±0.12	0.99±0.13	6.73±0.23	218.71±3.63	45.76±3.29[b]
A/S1/2+ H1/4	-0.18±0.05	-0.13±0.08	-0.24±0.04	-0.50±0.03	1.46±0.12	7.00±0.24	196.76±5.10	67.70±3.73[c]

Alphapet difference was significant at 0.05 level. Δ = Mean log change viable cell counts at 2, 4 , 6 , 8 and 24 hours, respectively.
$AUBKC_{0-24}$ = Area under bacterial killing and regrowth curves for 24 hours. BA_{24} = Bacterolytic area for 24 hours

Table 4-13: Results of time-kill analyzes of 5 isolates of MRSA no. 17, 107, 234, 466, and 777 by the combination of 1/8 MIC ampicillin plus 1/2 MIC alcoholic extract of *H. odorata* ROXB. at various time.

Conc	No of strains for which the levels of 90% killing						No of strains regrowth		
	2 hr	4 hr	6 hr	8 hr	10 hr	24 hr	8 hr	10 hr	24 hr
Control	-	-	-	-	-	-	-	1	5
1/8 ampi	2	-	-	-	-	-	-	-	5
1/2 ampi	5	5	5	3	-	-	-	-	5
1/4 extract	-	-	-	-	-	-	-	-	5
1/4 extract	-	-	-	-	-	-	-	-	5
1/8 ampi + 1/2 extract	5	5	4	-	-	-	-	-	5
1/2 ampi + 1/4 extract	5	5	4	4	-	-	-	-	5

Antibacterial activities were observed from the time kill study. The comparative activities between the combinations of various MIC levels of both extract and ampicillin were summarized as followed :

The number of bacteria killed and the number of strains inhibited by1/8 MIC ampicillin alone and the combination of 1/8 MIC ampicillin plus 1/2 MIC alcoholic extract of *H. odorata* ROXB. were shown as followed of the number of bacteria killed by the combination of 1/2 MIC alcoholic extract of *H. odorata* ROXB. plus 1/8 MIC ampicillin [BA_{24} = 64.04 log CFU/ml·h] were significantly higher than the number of bacteria killed by 1/8 MIC ampicillin alone [BA_{24} =27.45 log CFU/ml·h] ($p<0.05$) (Table 4-9). In addition, 1/8 MIC ampicillin alone showed bacteriostatic activtity against 2 isolates (no. 107 and 466) at 2 hour of growth and the regrowth of 5 isolates (no. 17, 107, 234, 466, and 777) were observed at 24th hours. The combination of 1/2 MIC alcoholic extract of *H. odorata* ROXB. plus 1/8 MIC ampicillin showed 90% killing against 5 isolates (no.17, 107, 234, 466, 777) at 2 hour of growth, 5 isolates (no.17, 107, 234, 466, and 777) at 4 hour of growth and 4 isolates (no.17, 107, 466, and 777) at 6 hour of growth.The regrowth of 5 isolates (no. 17, 107, 234, 466, and 777) were observed at 24th hours (Table 4-13).

The number of bacteria killed the number of strains inhibited by 1/2 MIC ampicillin alone and the combination of 1/2 MIC ampicillin plus 1/4 MIC alcoholic extract of H. odorata ROXB were shown as followed of the number of bacteria killed by the combination of 1/4 MIC alcoholic extract of *H.odorata* ROXB plus 1/2 MIC ampicillin [BA_{24} = 74.84 log CFU/ml·h] were not significantly higher than the number killed by 1/2 MIC ampicillin alone. [BA_{24} = 68.92 log CFU/ml·h] ($p>0.05$) (Table 4-9). In addition, 1/2 MIC ampicillin alone showed bacteriostatic activtity against 5 isolates (no. 17, 107, 234, 466, and 777) at 2 hour of growth , 5 isolates (no. 17, 107, 234, 466, and 777) at 4 hour of growth , 5 isolates (no. 17, 107, 234, 466, and 777) at 6 hour of growth , 3 isolates (no.107, 234, and 466) at 8 hour of growth, respectively. The regrowth of 5 isolates (no. 17, 107, 234, 466, and 777) were observed at 24^{th} hours. The combination of 1/4 MIC alcoholic extract of *H. odorata* ROXB plus 1/2 MIC ampicillin showed 90% killing against 5 isolates (no. 17, 107, 234, 466, and 777), at 2 hour of growth, 5 isolates (no. 17, 107, 234, 466, and 777), at 4 hour of growth,4 isolates (no. 17, 107, 234, and 466) at 6 hour of growth, 4 isolates (no. 17, 107, 234, 466) at 8 hour of growth. The regrowth of 5 isolates (no. 17, 107, 234, 466, and 777) were observed at 24^{th} hours (Table 4-13).

Table 4-14: Results of time-kill analyzes of 6 isolates of MRSA no. 20, 23, 32, 266, 268, and 777 by the combination of 1/4 MIC ampicillin plus 1/2 MIC alcoholic extract of *H. odorata* ROXB. at various time.

Conc	No of strains for which the levels of 90% killing						No of strains regrowth		
	2 hr	4 hr	6 hr	8 hr	10 hr	24 hr	8 hr	10 hr	24 hr
Control	-	-	-	-	-	-	-	-	6
¼ ampi	3	1	-	-	-	-	-	-	6
½ ampi	6	6	5	1	-	-	-	-	6
¼ extract	-	-	-	-	-	-	-	-	6
½ extract	-	-	-	-	-	-	-	-	6
¼ ampi + ½ extract	6	6	3	-	-	-	-	-	6
½ ampi + ¼ extract	6	6	4	2	-	-	-	-	6

Antibacterial activities were observed from the time kill study. The comparative activities between the combinations of various MIC levels of both extract and ampicillin were summarized as followed :

The number of bacteria killed and the number of strains inhibited by 1/4 MIC ampicillin alone and the combination of 1/4 MIC ampicillin plus 1/2 MIC alcoholic extract of *H. odorata* ROXB were shown as followed of the number of bacteria killed by the combination of 1/2 MIC alcoholic extract of *H. odorata* ROXB plus 1/4 MIC ampicillin [BA_{24} = 43.77 log CFU/ml·h] were significantly higher than the number killed by 1/4 MIC ampicillin alone [BA_{24} = 18.43 log CFU/ml·h] ($p<0.05$) (Table 4-10). In addition, ampicillin 1/4 MIC alone showed bacteriostatic activtity against 3 isolates (no. 266, 268, and 384) and 1 isolate (no.268) at 2, 4 hour of growth, respectively. The regrowth of 6 isolates (no. 20,23, 32, 266, 268, and 384) were observed at 24th hours. The combination of 1/2 MIC alcoholic extract of *H. odorata* ROXB plus 1/4 MIC ampicillin show 90% killing against 6 isolates (no. 20,23, 32, 266, 268, and 384), at 2 hour of growth, 6 isolates (no. 20,23, 32, 266, 268, and 384), at 4 hour of growth and 3 isolates (no 32, 266, and 268) at 6 hour of growth. The regrowth of 6 isolates (no. 20,23, 32, 266, 268, and 384) were observed at 24th hours (Table 4-14).

The number of bacteria killed and the number of strains inhibited by 1/2 MIC ampicillin alone and the combination of 1/2 MIC ampicillin plus 1/4 MIC alcoholic extract of *H. odorata* ROXB. were shown as followed of : the number of bacteria killed by the combination of 1/4 MIC alcoholic extract of *H. odorata* ROXB. plus 1/2 MIC ampicillin [BA_{24} = 53.97 log CFU/ml·h] were not significantly higher than the number killed by 1/2 MIC ampicillin alone [BA_{24} = 47.43 log CFU/ml·h] ($p>0.05$) (Table 4-10). In addition, 1/2 MIC ampicillin alone showed bacteriostatic activtity against 6 isolates (no. 20, 23, 32, 266, 268, and 384) at 2 hour of growth , 6 isolates (no. 20,23, 32, 266, 268, and 384) at 4 hour of growth against 5 isolates (no. 20, 23, 266, 268, and 384) and 1 isolate (no.384) at 6, 8 hour of growth, respectively. The regrowth of 6 isolates (no. 20, 23, 32, 266, 268, and 384) were observed at 24th hours. The combination of alcoholic extract of *H.odorata* ROXB 1/4 MIC plus ampicillin 1/2 MIC show 90% killing against 6 isolates (no. 20,23, 32, 266, 268, and 384), at 2 hour of growth, 6 isolates (no. 20,23, 32, 266, 268, and 384), at 4 hour of growth and 4 isolates (no.23, 266, 268, and 384), 2 isolates (no.266, and 384) at 6 ,8 hour of

growth, respectively. The regrowth of 6 isolates (no. 20, 23, 32, 266, 268, and 384) were observed at 24th hours (Table 4-14).

Table 4-15: Results of time-kill analyzes of 4 isolates of MRSA no. 20, 23, 31, and 32 by the combination of 1/8 MIC ampicillin/sulbactam plus 1/2 MIC alcoholic extract of *H. odorata* ROXB. at various time.

Conc	No of strains for which the levels of 90% killing						No of strains regrowth		
	2 hr	4 hr	6 hr	8 hr	10 hr	24hr	8 hr	10 hr	24 hr
Control	-	-	-	-	-	-	-	-	4
1/8 A/S	3	1	-	-	-	-	-	-	4
1/2 A/S	4	4	4	1	-	-	-	-	4
1/4 extract	-	-	-	-	-	-	-	-	4
1/2 extract	-	-	-	-	-	-	-	-	4
1/8 A/S + 1/2 extract	4	3	1	-	-	-	-	-	4
1/2 A/S + 1/4 extract	4	4	4	3	-	-	-	-	4

Antibacterial activities were observed from the time kill study. The comparative activities between the combinations of various MIC levels of both extract and ampicillin/sulbactam were summarized as followed :

The number of bacteria killed and the number of strains inhibited by 1/8 MIC ampicillin/sulbactam alone and the combination of 1/8 MIC ampicillin/sulbactam plus 1/2 MIC alcoholic extract of *H. odorata* ROXB. were shown as followed: the number of bacteria killed by the combination of 1/2 MIC alcoholic extract of *H. odorata* ROXB. plus 1/8 MIC ampicillin/sulbactam [BA_{24} =37.75 log CFU/ml·h] were significantly higher than the number killed by 1/8 MIC ampicillin/sulbactam alone [BA_{24} = 17.64 log CFU/ml·h] ($p<0.05$) (Table 4-11). In addition, 1/8 MIC ampicillin/sulbactam alone showed bacteriostatic activtity against 3 isolates (no.20, 23, and 32), 1 isolate (no.23) at 2, 4 hour of growth, respectively. The regrowth of 4 isolates (no.20, 23, 31, and 32) were observed at 24 hours. The combination of 1/2 MIC alcoholic extract of *H. odorata* ROXB. plus 1/8 MIC ampicillin/sulbactam

showed 90% killing against 4 isolates (no. 20, 23, 31, and 32), 3 isolates (no.23, 31, and 32), 1 isolate (no.23) at 2, 4 ,6 hour of growth, respectively. The regrowth of 4 isolates (no. 20, 23, 31, and 32) were observed at 24 hours (Table 4-15).

The number of bacteria killed and the number of strains inhibited by 1/2 MIC ampicillin/sulbactam alone and the combination of 1/2 MIC ampicillin/sulbactam plus 1/4 MIC alcoholic extract of *H. odorata* ROXB. were shown as followed of the number of bacteria killed by the combination of 1/4 MIC alcoholic extract of *H. odorata* ROXB. plus 1/2 MIC ampicillin/sulbactam [BA_{24} = 68.86 log CFU/ml·h] were not significantly higher than the number killed by 1/2 MIC ampicillin/sulbactam alone [BA_{24} = 78.70 log CFU/ml·h] ($p>0.05$) (Table4-11). In addition, 1/2 MIC ampicillin/sulbactm alone showed bacteriostatic activtity against 4 isolates (no. 20, 23, 31, and 32) at 2 hour of growth ,4 isolates (no. 20, 23, 31, and 32) at 4 hour of growth , 4 isolates (no. 20, 23, 31, and 32) at 6 hour of growth and 1isolate (no.32) at 8 hour of growth. The regrowth of 4 isolates (no. 20, 23, 31, and 32) were observed at 24th hours. The combination of 1/4 MIC alcoholic extract of *H. odorata* ROXB. plus 1/2 MIC ampicillin/sulbactam showed 90% killing against 4 isolates (no. 20, 23, 31, and 32) at 2 hour of growth, 4 isolates (no. 20, 23, 31, and 32) at 4 hour of growth, 4 isolates (no. 20, 23, 31, and 32) at 6 hour of growth and 3 isolates (no.20, 23, and 31) at 8 hour of growth. The regrowth of 4 isolates (no. 20, 23, 31, and 32) were observed at 24th hours (Table 4-15).

Table 4-16: Results of time-kill analyzes of 7 isolates of MRSA no. 3, 94, 102, 279, 777, 786, and 1028 by the combination of 1/4 MIC ampicillin/sulbactam plus 1/2 MIC alcoholic extract of *H. odorata* ROXB. at various time.

Conc	No of strains for which the levels of 90% killing						No of strains regrowth		
	2 hr	4 hr	6 hr	8 hr	10 hr	24hr	8 hr	10 hr	24 hr
Control	-	-	-	-	-	-	-	-	7
¼ A/S	5	5	1	-	-	-	-	-	7
½ A/S	7	7	6	3	-	-	-	-	7
¼ extract	-	-	-	-	-	-	-	-	7
½ extract	-	-	-	-	-	-	-	-	7
¼ A/S + ½ extract	7	7	5	-	-	-	-	-	7
½ A/S + ¼ extract	7	7	7	7	-	-	-	-	7

Antibacterial activities were observed from the time kill study. The comparative activities between the combinations of various MIC levels of both extract and ampicillin were summarized as followed :

The number of bacteria killed and the number of strains inhibited by 1/4 MIC ampicillin/sulbactam alone and the combination of ¼ MIC ampicillin/sulbactam plus 1/2 MIC Alcoholic extract of *H. odorata* ROXB. were shown as followed : the number of bacteria killed by the combination of 1/2 MIC alcoholic extract of *H. odorata* ROXB. plus 1/4 MIC ampicillin/sulbactam [BA_{24} = 45.76 log CFU/ml·h] were significantly higher than the number killed by 1/4 MIC ampicillin/sulbactam alone [BA_{24} = 25.75 log CFU/ml·h] ($p<0.05$) (Table 4-12). In addition, 1/4 MIC ampicillin/sulbactam alone showed bacteriostatic activtity against 5 isolates (no.94, 279, 777, 786, and 1028) at 2 hour of growth , 5 isolates (no.94, 279, 777, 786, and 1028)at 4 hour of growth and 1 isolates (no.777) at 6 hour of growth. The regrowth of 7 strains(no.3, 94, 102, 107, 279, 777, 786, and 1028) were observed at 24th hours. The combination of ½ MIC Alcoholic extract of *H. odorata* ROXB. plus 1/4 MIC ampicillin/sulbactam show 90% killing against 7 isolates (no.3, 94, 102, 279, 777, 786, and 1028) at 2 hour of growth, 7 isolates (no.3, 94, 102, 279, 777, 786, and

1028) at 4 hour of growth and 5 isolates (no 94, 102, 279, 786, and 1028) at 6 hour of growth. The regrowth of 7 isolates (no.3, 94, 102, 279, 777, 786, and 1028) were observed at 24 hours (Table 4-16).

The number of bacteria killed and the number of strains inhibited by 1/2 MIC ampicillin/sulbactam alone and the combination of 1/2 MIC ampicillin/sulbactam plus 1/4 MIC alcoholic extract of *H. odorata* ROXB. were shown as followed of the number of bacteria killed by the combination of 1/4 MIC alcoholic extract of *H. odorata* ROXB. plus 1/2 MIC ampicillin [BA_{24} = 67.70 log CFU/ml·h] were not significantly higher than the number killed by 1/2 MIC ampicillin alone [BA_{24} = 66.89 log CFU/ml·h] ($p>0.05$) (Table 4-12). In addition, 1/2 MIC ampicillin/sulbactm alone showed bacteriostatic activtity against 7 isolates (no.3, 94, 102, 279, 777, 786, and 1028) at 2hour of growth ,7 isolates (no.3, 94, 102, 279, 777, 786, and 1028) at 4 hour of growth , 6 isolates (no.3, 102, 279, 777, 786, and 1028), 3 isolates (no.279, 777, and 1028) at 6 hour of growth, 6 isolates (no.3, 102, 279, 777, 786, and 1028), 3 isolates (no.279, 777, and 1028) at 8 hour of growth, respectively. The regrowth of 7 isolates (no.3, 94, 102, 279, 777, 786, and 1028) were observed at 24^{th} hours. The combination of 1/4 MIC alcoholic extract of *H. odorata* ROXB. plus 1/2 MIC ampicillin show 90% killing against 7 isolates (no.3, 94, 102, 279, 777, 786, and 1028) at 2 hour of growth ,7 isolates (no.3, 94, 102, 279, 777, 786, and 1028) at 4 hour of growth, 7 isolates (no.3, 94, 102, 279, 777, 786, and 1028) at 6 hour of growth and 7 isolates (no.3, 94, 102, 279, 777, 786, and 1028) at 8 hour of growth. The regrowth of 7 isolates (no.3, 94, 102, 279, 777, 786, and 1028) were observed at 24^{th} hours (Table 4-16).

CHAPTER V

DISCUSSION &CONCLUSION

The prevalence of MRSA has increased in many parts of the world. Because MRSA isolates do not resist to only β-lactam antibiotics, but also resist to macrolides, licosamides and fluoroquinolones. Furthermore, in spite of recent report of vancomycin resistant *Staphylococcus aureus* (VRSA), vancomycin is still remained the drug of choice for most MRSA-associated diseases (Smith et al., 1999) . Thus, the cost of treatment is high and becomes the problem in our health care system, so the concerted efforts have again been made to find antimicrobial materials from natural products and traditional medicines. Over the last two decades, several resveratrol oligomers, the stilbene derivatives (polyphenolic compound), have been isolated from Dipterocarpaceae plants. Zgoda-Pols et al., (2002). reported the resveratrol tetramers, hopeaphenol A and vaticaphenol A, which were isolated from stem bark of *Vatica oblongifolia* spp. *oblongifolia* had moderate activity against MRSA. Many Dipterocarpaceae plants are grown in Thailand including *H. odorata* ROXB. The result in this study showed that alcoholic extract of *H. odorata* ROXB. had inhibitory effect to all 30 isolates of MRSA by disc diffusion method and agar dilution method. In addition, it was also shown that the active component in the alcoholic extract of *H .odorata* ROXB. was tannin, which was widely known as a polyphenolic compound. Many studies reported that polyphenolic compound, such as tannins (Scalbert, 1991; Stern et al., 1996 ; and Schultz, 1988) and flavonoids (Rojas et al., 1992; Perrett et al., 1995) with different structures could inhibit microbial growth *in vitro* (Chung et al., 1998). These compounds could bind to the proteins and cause the coagulation of various proteins (White, 1987). Thus, the inhibitory activity of the extract against MRSA might occur from the tannin binding to certain proteins in MRSA including penicillin binding protein (PBPs) and certain β-lactamase enzymes leading to inactivation of proteins and loss of resistant function.

At present, ampicillin is readily inactivated by β-lactamase enzymes from the pathogens and is useless in the treatment of infections caused by *S. aureus* or other organisms producing such enzymes. Increasing resistance appeared not only in the strains of *S. aurues* but also in the strains of *Streptococcus pneumoniae, Neisseria gonorrhea*, and nontyphoidal *Salmonella*. Methicillin resistance in *S. aureus* causes

by the production of PBP2a which has low affinity to most β-lactam antibiotics. However, certain β-lactams, including ampicillin, piperacillin and imipenem, have stronger binding affinities for PBP2a than other antibiotics (Chambers et al, 1990). Many β-lactam/β-lactammase inhibitors including ampicillin-sulbactam, ticarcillin-clavulanate, and piperacillin-tazobactam have been used in the treatment of infections caused by β-lactamase producing pathogens for years. The combination between ampicillin and sulbactam are commonly used to treat soft tissue and urinary tract infection. Many evidences also support the possible treatment of MRSA infection with ampicillin/sulbactam. Because most MRSA strains produce β-lactamase (Norris et al., 1994) and the synergistic effect between sulbactam and β-lactam antibiotics against MRSA has been shown.(Kobayashi et al., 1989). In this study, the MICs of ampicillin in ampicillin/sulbactam was lower than MICs of ampicillin alone by 2-4 folds.

Zhi-Qing Hu et al. reported that epigallocatechin gallate (EGCg), which is tannin compound also showed anti-MRSA activity with an MIC of 100 μg/ml. When ampicillin/sulbactam was combined with EGCg at sub-MICs, the MIC_{50} of ampicillin/sulbactam was decreased from 16-32 to 8 μg/ml in the presence of 6.25 μg/ml (1/16 MIC) of EGCg and the MIC_{90} of ampicillin/sulbactam decreased to 4 μg/ml in the presence of 25 μg/ml (1/4 MIC) of EGCg.(Zhi-Qing Hu et al., 2001) In this study, the MIC of ampicillin in the combination of ampicillin plus 1/2 MIC of the alcoholic extract of *H. odorata* Roxb was decreased from MIC of ampicillin alone by 2-8 folds when tested against all 30 isolates of MRSA (100%). However, when the concentration of the extract was decrease to 1/4 MIC, the MIC of ampicillin was decreased by 2-4 folds as compared to the MIC of ampicillin alone against only 14 strains (46.67%). Meanwhile, when ampicillin/sulbactam was combined to 1/2 MIC of the alcoholic extract *H. odorata* ROXB, the MIC of ampicillin/sulbactam was decreased by 2-16 folds against all 30 isolates of MRSA (100%) as compared to the MIC of ampicillin/sulbactam alone. Additionally, the MIC of ampicillin/sulbactam in this combination were lower than the susceptibility breakpoint (≤ 8 μg/ml), against all 30 isolates. When ampicillin/sulbactam was combined to the 1/4 MIC of the alcoholic extract *H. odorata* ROXB, the MIC was decreased 2-4 folds against only 12 isolates (40%) as compared to the MIC of ampicillin alone. The study of synergistic interaction between alcoholic extract of 1/2 MIC or 1/4 MIC *H. odorata* ROXB. plus

ampicillin was performed by checkerboard method. It was showed that most of the strains (73.33%) were inhibited by the partial synergistic action between ampicillin and 1/2 MIC *H. odorata* ROXB while lower number of isolates were inhibited when the concentration of the extract was lower to 1/4 MIC. Similar result was obtained when tested with the combination of the alcoholic extract of *H. odorata* ROXB. plus ampicillin/sulbactam. Thus, further study by Time-kill method was performed using the combination between the 1/2 MIC of the alcoholic extract *H. odorata* ROXB. and ampicillin or ampicillin/sulbactam. It was shown that the 90% killing of the strains were significantly higher by the combination than by ampicillin or ampicillin/sulbactam alone in the same dose ($p<0.05$). All combination activity of alcoholic extract of *H. odorata* ROXB. plus ampicillin or ampicillin/sulbactam showed bacteriostatic greater than alcoholic extract of *H. odorata* ROXB., ampicillin or ampicillin/sulbactam alone. The combination between ampicillin and the alcoholic extract *H. odorata* ROXB. had better synergistic effect than the effect of combination between ampicillin and sulbactam because of the compound in the extract not only inhibit the function of cell wall protein but may also inhibit the β-lactamase enzyme. Since the antibacterial effect of the combination between ampicillin and the extract and the combination between ampicillin/sulbactam and the extract was not different. The results from this part of the study indicated that the alcoholic extract of *H. odorata* ROXB. was synergist to β-lactam antibiotics tested. As already mentioned above, the extract contained tannins could possibly bind to the microbial cell wall proteins and also β-lactamase enzymes and cause protein coagulation which led to the inactivation of such proteins function. (White, 1987; Scalbert, 1991; Stern et al., 1996 ; Schultz, 1988). In the combination, the synergistic effect may occur from the decrease in cell wall intregity by tannin along with the inhibition of new cell wall synthesis by ampicillin.

Thus, suggestive informations on the usefulness of Thai Herbal medicine has been provided in this study. The alcoholic extract of *H. odorata* ROXB., the commonly found herb in our country could be used in the combination with simple antibiotic such as ampicillin and caused the decrease in the MIC of ampicillin. However, further studies in various aspects are needed including the purification and identification of active compound(s) in the plant extract, the inhibitory effect of pure tannins and the *in vivo* studies on the effect of these pure compounds to obtain more conclusive evidences in the new drug discovery.

Conclusion

Preliminary informations obtained from this study indicated that the alcoholic extract of *H. odorata* Roxb. was a very interesting crude drug for further steps in new drug discovery and might be used in a combination with the antimicrobials. The antibacterial activity of the combinations between the extract and ampicillin or ampicillin/sulbactam were better than the antibacterial activity of each drug. In addition, the plant extract showed the partial synergistic effect to both ampicillin and ampicillin/sulbactam and could also lower the MICs of both drugs. Thus, the combinations of the extract and ampicillin or ampicillin/sulbactam could be promising alternatives in the treatment of infections due to MRSA that were resistant to ampicillin and ampicillin/sulbactam.

The *in vitro* study demonstrated that alcoholic extract of *H. odorata* ROXB. plus ampicillin or plus ampicillin/sulbactam was effective in combating against methicillin-resisitant *Staphylococcus aureus*. This study is one of the few studies using the combination of crude extract of Thai herbal medicine with antimicrobial agents in Thailand. Even though, it was shown that *H. odorata* ROXB. could be a source of new antibiotic compounds but further studies are still needed including the purification of active constituents from the extracts studied and the test for specific antimicrobial activity. It would also be important to test for the action of the extract on the other antibiotic resistant pathogens or other antimicrobial combinations to gain further insight into the specificity of the antimicrobial action and undergo further pharmacological evaluation.

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APPENDICES

Table A-1: Raw data of sensitivity of alcoholic extract of *H. odorata* ROXB. against 30 isolates by disk diffusion method and beta-lactamase activity.

No.	*S.aureus* strain No.	Concentration of alcoholic extract of *H.odorata* ROXB 0.02 mg/disk	Betalactamase activity
		Inhibition zone (mm)	
1	3	7.26	+
2	9	7.44	+
3	17	6.96	+
4	19	6.48	+
5	20	7.28	+
6	23	6.73	+
7	31	7.26	+
8	32	7.65	+
9	34	6.42	+
10	38	8.40	+
11	94	8.35	+
12	102	7.64	+
13	107	7.69	+
14	152	9.40	+
15	200	NZ	+
16	216	6.52	+
17	234	7.54	+
18	240	7.13	+
19	241	6.95	+
20	266	7.42	+
21	268	7.25	+
22	269	7.16	+
23	279	6.81	+
24	384	9.40	+
25	466	6.83	+
26	643	9.28	-
27	777	7.88	+
28	786	6.56	+
29	F9	8.36	+
30	1028	10.08	+

NZ = no inhibition zone

Table A-2: Raw data of susceptibility testing by agar dilution method.

MRSA (strain)	Alcoholic extract of *H. odorata* ROXB (μg/ml)	Ampicillin (μg/ml)	Ampi/sul (μg/ml)	Oxacillin (μg/ml)
ATCC 29213	125	0.5	0.5	0.25
3	125	64	32	256
9	125	64	32	> 256
17	62.5	64	32	> 256
19	125	64	32	> 256
20	125	64	16	> 256
23	125	64	16	> 256
31	125	32	16	>256
32	125	64	16	> 256
34	125	64	32	> 256
38	125	128	32	>256
94	125	128	32	>256
102	125	64	32	> 256
107	62.5	64	32	> 256
152	62.5	64	32	> 256
200	125	64	32	> 256
216	125	128	32	> 256
234	125	128	32	>256
240	125	64	32	>256
241	125	64	32	> 256
266	125	64	16	>256
268	125	32	16	> 256
269	125	64	16	> 256
279	125	128	32	> 256
384	125	64	32	>256
466	125	128	32	> 256
643	125	16	32	> 256
777	125	32	32	> 256
786	62.5	64	32	> 256
F9	125	64	32	> 256
1028	62.5	8	8	256

Table A-3: Log viable cell counts at time point in 18 isolates of MRSA.

isolates no.	Antimicrobial agents	Log viable count (log CFU/ml) at time point						
		0	2	4	6	8	10	24
3	control	6.53	7.07	7.83	8.37	9.75	11.39	16.19
	Ampi/sul(1/4 MIC)	6.60	6.93	7.11	8.20	8.64	10.30	15.44
	Ampi/sul(1/2 MIC)	6.50	6.30	5.81	5.70	6.20	7.71	13.65
	Extract (1/4 MIC)	6.51	6.92	7.37	8.48	9.65	10.62	15.72
	Extract (1/2 MIC)	6.55	6.79	7.00	7.92	8.53	9.51	15.36
	Ampi/sul (1/4 MIC) +Extract (1/2 MIC)	6.34	6.21	6.00	6.82	7.33	8.68	14.72
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	6.54	6.06	5.92	5.76	5.40	6.75	13.76
17	control	6.18	6.68	7.60	8.71	9.66	11.03	16.58
	Ampicillin(1/8 MIC)	6.17	6.28	7.14	8.69	9.58	10.66	15.67
	Ampicillin(1/2 MIC)	6.25	6.22	5.95	5.46	6.70	7.22	14.85
	Extract (1/4 MIC)	6.17	6.98	7.69	8.70	9.60	10.37	15.60
	Extract (1/2 MIC)	6.15	6.75	7.33	8.00	8.53	9.50	15.84
	Ampicillin(1/8 MIC) +Extract (1/2 MIC)	6.11	5.97	5.80	5.40	6.44	8.24	14.66
	Ampicillin(1/2 MIC) +Extract (1/4 MIC)	6.18	5.88	5.55	5.39	5.00	6.71	13.72
20	control	6.15	6.67	7.97	8.52	9.59	10.70	16.27
	Ampicillin(1/4 MIC)	6.00	6.07	6.19	7.30	8.56	9.52	15.26
	Ampicillin(1/2 MIC)	6.86	6.22	6.04	5.95	6.54	7.38	13.58
	Ampi/sul(1/8 MIC)	6.46	6.04	6.30	7.70	8.93	9.70	15.59
	Ampi/sul(1/2 MIC)	6.37	6.34	5.60	5.24	5.00	5.99	13.78
	Extract (1/4 MIC)	6.41	6.76	7.20	7.37	8.48	9.99	15.63
	Extract (1/2 MIC)	6.44	6.76	6.85	7.40	7.76	8.44	14.35
	Ampicillin(1/4 MIC) +Extract (1/2 MIC)	6.76	6.48	6.21	7.80	8.15	9.40	15.54
	Ampicillin(1/2 MIC) +Extract (1/4 MIC)	6.38	6.09	5.92	6.13	7.05	8.65	15.11
	Ampi/sul (1/8 MIC) +Extract (1/2 MIC)	6.70	6.20	7.10	7.22	8.94	9.74	15.59
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	6.14	5.99	5.65	5.30	5.00	5.72	13.53
23	control	6.19	6.80	7.00	7.89	9.10	10.72	15.60
	Ampicillin(1/4 MIC)	6.33	6.85	7.13	7.94	8.89	10.88	15.55
	Ampicillin(1/2 MIC)	6.44	6.19	6.17	6.08	7.84	8.93	14.98
	Ampi/sul(1/8 MIC)	6.35	6.28	6.11	7.08	8.72	9.80	14.26
	Ampi/sul(1/2 MIC)	6.40	6.18	6.02	5.98	5.20	6.76	13.10
	Extract (1/4 MIC)	6.30	6.57	6.74	7.84	8.39	9.86	15.13
	Extract (1/2 MIC)	6.24	6.74	6.89	6.94	7.76	8.89	15.09
	Ampicillin(1/4 MIC) +Extract (1/2 MIC)	6.35	6.25	6.03	6.81	7.83	8.92	15.37
	Ampicillin(1/2 MIC) +Extract (1/4 MIC)	6.24	6.18	6.10	5.89	6.70	8.20	14.20
	Ampi/sul(1/8 MIC) +Extract(1/2 MIC)	6.74	6.65	6.60	6.09	7.68	9.76	15.08
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	6.18	6.04	5.95	5.74	5.09	6.24	13.30

Table A-3: (continue) Log viable cell counts at time point in 18 isolates of MRSA.

isolates no.	Antimicrobial agents	Log viable count (log CFU/ml) at time point						
		0	2	4	6	8	10	24
31	control	6.35	7.11	8.51	9.49	10.66	11.95	16.57
	Ampi/sul(1/8 MIC)	6.38	6.53	7.07	8.24	9.72	11.61	15.75
	Ampi/sul(1/2 MIC)	6.35	6.25	5.96	5.77	6.51	7.69	14.50
	Extract (1/4 MIC)	6.35	6.40	7.19	8.39	9.66	11.75	15.27
	Extract(1/2 MIC)	6.38	6.34	6.88	7.58	8.10	10.65	15.09
	Ampi/sul(1/8 MIC) +Extract(1/2 MIC)	6.29	6.04	5.81	6.23	7.33	8.58	14.75
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	6.38	6.09	5.48	5.33	5.27	6.62	13.23
32	control	6.46	6.54	7.39	8.44	9.54	10.57	16.92
	Ampicillin(1/4 MIC)	6.47	6.16	6.85	7.37	8.55	9.65	16.63
	Ampicillin(1/2 MIC)	6.90	6.16	6.10	7.11	8.37	9.32	15.41
	Ampi/sul(1/8 MIC)	6.34	6.16	7.39	8.31	9.50	10.30	15.34
	Ampi/sul(1/2 MIC)	6.35	6.14	5.94	5.45	5.33	6.36	14.59
	Extract (1/4 MIC)	6.22	6.78	7.12	8.94	9.72	10.09	15.15
	Extract (1/2 MIC)	6.14	6.90	7.37	8.15	9.00	9.37	15.47
	Ampicillin(1/4 MIC) +Extract (1/2 MIC)	6.28	6.16	6.09	6.08	6.46	7.23	15.64
	Ampicillin(1/2 MIC) +Extract (1/4 MIC)	6.14	6.07	5.90	6.45	7.42	8.41	15.69
	Ampi/sul(1/8 MIC) +Extract(1/2 MIC)	6.29	6.24	6.09	7.31	8.30	9.25	15.75
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	6.43	6.26	6.16	5.88	6.37	6.37	13.70
94	control	6.52	6.85	7.34	8.28	9.60	9.71	16.27
	Ampi/sul(1/4MIC)	6.38	6.28	6.10	6.57	7.32	8.66	15.45
	Ampi/sul(1/2 MIC)	6.31	6.10	5.89	5.55	5.00	5.66	12.16
	Extract (1/4 MIC)	6.44	6.45	6.84	7.28	8.39	8.43	14.29
	Extract(1/2 MIC)	6.13	6.49	6.89	7.30	8.00	8.71	14.36
	Ampi/sul(1/4 MIC) +Extract (1/2 MIC)	6.27	6.09	5.86	5.57	6.18	7.26	14.38
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	6.50	6.33	6.05	5.75	5.26	6.43	12.50
102	control	6.13	6.40	7.76	8.80	9.78	10.86	15.50
	Ampi/sul(1/4MIC)	6.31	6.46	6.94	7.74	8.70	9.85	15.49
	Ampi/sul(1/2 MIC)	6.24	6.02	5.93	5.55	5.06	6.86	13.25
	Extract (1/4 MIC)	6.02	6.18	6.64	7.62	8.64	9.97	14.65
	Extract(1/2 MIC)	6.27	6.33	6.83	7.17	7.22	8.44	14.68
	Ampi/sul(1/4 MIC) +Extract (1/2 MIC)	6.17	6.14	5.84	5.42	6.47	7.47	14.36
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	6.20	6.10	6.00	5.75	5.11	6.70	13.61
107	control	6.18	6.85	7.44	8.62	9.11	11.92	16.15
	Ampicillin(1/8MIC)	6.24	6.20	6.58	7.09	8.85	9.13	15.29
	Ampicillin(1/2 MIC)	6.22	6.09	6.00	5.84	5.20	6.10	14.39
	Extract (1/4 MIC)	6.40	6.62	6.95	7.66	8.06	9.72	15.60
	Extract(1/2 MIC)	6.35	6.42	6.89	7.26	7.85	9.34	14.38
	Ampicillin(1/8 MIC) +Extract (1/2 MIC)	6.11	6.06	5.93	5.76	6.55	7.10	14.13
	Ampicillin(1/2 MIC) +Extract(1/4 MIC)	6.22	6.10	5.94	5.35	5.04	6.40	13.26

Table A-3:(continue) Log viable cell counts at time point in 18 isolates of MRSA.

isolates no.	Antimicrobial agents	Log viable count (log CFU/ml) at time point						
		0	2	4	6	8	10	24
234	control	6.29	6.47	7.02	8.74	9.34	11.66	16.15
	Ampicillin(1/8MIC)	6.32	6.40	6.93	7.27	8.90	9.65	15.08
	Ampicillin(1/2 MIC)	6.20	6.03	5.83	5.40	5.17	6.66	14.34
	Extract (1/4 MIC)	6.08	6.31	6.56	7.05	8.72	8.52	15.05
	Extract(1/2 MIC)	6.39	6.42	6.93	7.25	7.66	8.26	15.24
	Ampicillin(1/8 MIC) +Extract (1/2 MIC)	6.14	5.84	5.44	6.22	6.68	7.21	14.10
	Ampicillin(1/2 MIC) +Extract(1/4 MIC)	6.09	5.99	5.42	5.32	4.99	5.71	13.46
266	control	6.28	6.60	7.18	8.76	9.22	10.95	15.50
	Ampicillin(1/4MIC)	6.37	6.24	7.08	7.89	8.62	9.58	15.31
	Ampicillin(1/2 MIC)	6.20	6.03	5.83	5.65	6.32	7.49	14.39
	Extract (1/4 MIC)	6.15	6.66	7.42	8.37	9.00	9.70	15.17
	Extract(1/2 MIC)	6.37	6.72	7.33	8.11	8.95	9.27	15.06
	Ampicillin(1/4 MIC) +Extract (1/2 MIC)	6.25	6.08	5.89	5.45	6.18	7.49	14.16
	Ampicillin(1/2 MIC) +Extract(1/4 MIC)	6.11	5.95	5.53	5.24	5.10	6.45	13.95
268	control	6.15	6.70	7.17	8.97	9.66	10.36	15.78
	Ampicillin(1/4MIC)	6.27	6.13	6.05	6.83	8.44	9.17	15.03
	Ampicillin(1/2 MIC)	6.31	6.16	6.05	5.85	6.10	7.75	14.49
	Extract (1/4 MIC)	6.13	6.50	6.75	7.28	8.83	9.11	15.46
	Extract(1/2 MIC)	6.04	6.27	6.53	7.16	7.69	8.03	15.37
	Ampicillin(1/4 MIC) +Extract (1/2 MIC)	6.09	6.03	5.83	5.51	6.53	7.07	14.40
	Ampicillin(1/2 MIC) +Extract(1/4 MIC)	6.31	6.18	5.88	5.44	5.44	6.25	13.32
279	control	6.39	6.74	7.51	8.66	9.58	10.91	15.79
	Ampi/sul(1/4MIC)	6.29	6.18	6.00	6.87	7.12	8.53	14.72
	Ampi/sul(1/2 MIC)	6.37	6.23	5.94	5.75	5.33	6.68	13.85
	Extract (1/4 MIC)	6.32	6.82	7.58	7.90	8.63	8.90	14.65
	Extract(1/2 MIC)	6.40	6.72	6.93	7.61	7.95	8.73	14.57
	Ampi/sul(1/4 MIC) +Extract (1/2 MIC)	6.36	6.24	5.95	5.57	6.67	7.73	13.47
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	6.25	6.05	5.87	5.64	5.16	6.81	13.77
384	control	6.15	6.65	7.31	8.94	9.67	10.51	16.63
	Ampicillin(1/4MIC)	6.38	6.24	6.81	7.57	8.90	9.63	15.73
	Ampicillin(1/2 MIC)	6.51	6.32	5.94	5.64	5.31	6.74	13.57
	Extract (1/4 MIC)	6.27	6.71	7.02	7.88	8.65	8.93	14.78
	Extract(1/2 MIC)	6.40	6.69	6.91	7.60	7.87	8.54	14.45
	Ampicillin(1/4 MIC) +Extract (1/2 MIC)	6.28	6.01	5.89	6.64	6.96	7.64	13.57
	Ampicillin(1/2 MIC) +Extract(1/4 MIC)	6.45	6.09	5.84	5.61	5.13	6.68	13.89
466	control	6.27	6.34	7.71	8.59	9.23	10.24	15.60
	Ampicillin(1/8MIC)	6.16	6.03	6.37	6.83	7.50	8.33	14.48
	Ampicillin(1/2 MIC)	6.11	6.06	5.84	5.47	5.01	6.68	13.34
	Extract (1/4 MIC)	6.44	6.70	6.95	7.15	7.68	6.18	14.86
	Extract(1/2 MIC)	6.38	6.60	6.78	7.02	7.27	8.05	14.23
	Ampicillin(1/8 MIC) +Extract (1/2 MIC)	6.31	6.23	6.11	5.71	6.22	6.72	14.18
	Ampicillin(1/2 MIC) +Extract(1/4 MIC)	6.34	6.15	6.07	5.75	5.28	6.30	13.20

Table A-3:(continue) Log viable cell counts at time point in 18 isolates of MRSA.

isolates no.	Antimicrobial agents	Log viable count (log CFU/ml) at time point						
		0	2	4	6	8	10	24
777	control	6.30	6.39	7.37	8.27	9.84	10.37	16.38
	Ampicillin(1/8 MIC)	6.33	6.39	7.41	8.25	9.32	10.31	16.56
	Ampicillin(1/2 MIC)	6.32	6.30	6.25	6.11	6.48	7.40	15.46
	Ampi/sul(1/4 MIC)	6.44	6.35	6.24	6.16	6.35	7.36	15.63
	Ampi/sul(1/2 MIC)	6.39	6.34	6.05	5.88	5.12	6.41	14.40
	Extract (1/4 MIC)	6.35	6.27	7.15	7.40	8.58	9.47	16.69
	Extract (1/2 MIC)	6.36	6.44	6.57	7.33	8.44	9.31	16.62
	Ampicillin(1/8 MIC) +Extract (1/2 MIC)	6.24	6.21	6.41	6.27	7.16	8.09	15.65
	Ampicillin(1/2 MIC) +Extract (1/4 MIC)	6.35	6.28	6.25	6.47	7.39	8.45	15.57
	Ampi/sul(1/4 MIC) +Extract(1/2 MIC)	6.40	6.28	6.25	6.50	7.18	8.07	15.47
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	6.45	6.41	6.00	5.88	5.28	6.95	14.67
786	control	6.15	6.42	7.88	8.75	9.86	10.23	15.32
	Ampi/sul(1/4MIC)	6.34	6.13	6.45	7.33	8.90	9.38	14.50
	Ampi/sul(1/2 MIC)	6.30	6.11	6.05	5.77	5.15	6.30	13.23
	Extract (1/4 MIC)	6.04	6.28	6.82	7.19	7.84	8.12	15.69
	Extract(1/2 MIC)	6.01	6.15	6.66	7.13	7.65	8.11	15.09
	Ampi/sul(1/4 MIC) +Extract (1/2 MIC)	6.34	6.26	6.19	6.00	6.68	7.84	14.56
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	6.13	6.05	5.84	5.60	5.09	6.97	13.41
1028	control	6.51	6.86	7.02	7.95	8.67	9.05	16.75
	Ampi/sul(1/4MIC)	6.15	6.08	6.00	6.18	7.28	8.72	15.78
	Ampi/sul(1/2 MIC)	6.07	5.98	5.65	5.11	4.99	5.58	13.20
	Extract (1/4 MIC)	6.44	6.87	6.90	6.99	7.51	8.04	15.57
	Extract(1/2 MIC)	6.44	6.89	7.00	7.10	7.65	8.65	15.83
	Ampi/sul(1/4 MIC) +Extract (1/2 MIC)	6.09	6.02	5.63	5.19	6.68	7.04	14.22
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	6.13	5.97	5.74	5.39	4.95	5.88	13.76

Table A-4: Log change viable counts at time point and kinetic parameters in 18 isolates of MRSA.

isolates No.	Antimicrobial agent	Viable log change						AUBKC 0-24	Bacteriolytic Area
		Δ2	Δ4	Δ6	Δ8	Δ10	Δ24		
3	control	0.54	0.76	0.54	1.38	1.64	4.80	267.02	-
	Ampi/sul(1/4 MIC)	0.33	0.18	1.09	0.44	1.66	5.14	258.84	8.18
	Ampi/sul(1/2 MIC)	-0.20	-0.49	-0.11	0.50	1.51	5.94	211.75	55.27
	Extract (1/4 MIC)	0.41	0.45	1.11	1.17	0.97	5.10	266.35	0.67
	Extract (1/2 MIC)	0.24	0.21	0.92	0.61	0.78	5.85	250.63	16.39
	Ampi/sul (1/4 MIC) +Extract (1/2 MIC)	-0.13	-0.21	0.82	0.51	1.35	6.04	231.54	35.48
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	-0.48	-0.14	-0.16	-0.36	1.35	7.01	203.14	63.88
17	control	0.50	0.92	1.11	0.95	1.37	5.55	275.78	-
	Ampicillin(1/8 MIC)	0.11	0.86	1.55	0.89	1.08	5.01	264.52	11.26
	Ampicillin(1/2 MIC)	-0.03	-0.27	-0.49	1.24	0.52	7.63	216.62	59.16
	Extract (1/4 MIC)	0.81	1.01	1.01	0.90	0.77	5.23	264.57	11.21
	Extract (1/2 MIC)	0.60	0.58	0.67	0.53	0.97	6.34	254.25	20.75
	Ampicillin(1/8 MIC) +Extract (1/2 MIC)	-0.14	-0.17	-0.40	1.04	1.80	6.42	221.90	53.10
	Ampicillin(1/2 MIC) +Extract (1/4 MIC)	-0.30	-0.33	-0.16	-0.39	1.71	7.01	199.54	76.24
20	control	0.52	1.30	0.55	1.07	1.11	5.57	271.14	-
	Ampicillin(1/4 MIC)	0.07	0.12	1.11	1.26	0.96	5.74	245.22	25.92
	Ampicillin(1/2 MIC)	-0.64	-0.18	-0.09	0.59	0.84	6.20	210.46	60.68
	Ampi/sul(1/8 MIC)	-0.42	0.26	1.40	1.23	0.77	5.89	253.30	17.84
	Ampi/sul(1/2 MIC)	-0.03	-0.74	-0.36	0.24	0.99	7.79	195.11	76.03
	Extract (1/4 MIC)	0.35	0.44	0.17	1.11	1.51	5.64	255.36	15.78
	Extract (1/2 MIC)	0.32	0.09	0.55	0.36	0.68	5.91	231.95	39.19
	Ampicillin(1/4 MIC) +Extract (1/2 MIC)	-0.28	-0.27	1.59	0.35	1.25	6.14	248.02	23.12
	Ampicillin(1/2 MIC) +Extract (1/4 MIC)	-0.29	-0.17	0.21	0.92	1.60	6.46	231.73	39.41
	Ampi/sul (1/8 MIC) +Extract (1/2 MIC)	-0.50	0.90	0.12	1.72	0.80	5.85	252.67	18.47
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	-0.15	-0.34	-0.35	-0.30	0.72	7.81	190.49	80.65
23	control	0.61	0.20	0.89	1.21	1.62	4.88	262.73	-
	Ampicillin(1/4 MIC)	0.52	0.28	0.81	0.95	1.76	4.90	262.23	0.50
	Ampicillin(1/2 MIC)	-0.25	-0.02	-0.09	1.76	1.09	6.05	235.30	27.40
	Ampi/sul(1/8 MIC)	-0.07	-0.17	0.97	1.64	1.08	4.46	240.95	21.78
	Ampi/sul(1/2 MIC)	-0.22	-0.16	-0.04	0.78	1.56	6.34	198.84	63.79
	Extract (1/4 MIC)	0.27	0.17	1.10	0.55	1.47	5.27	250.17	12.56
	Extract (1/2 MIC)	0.50	0.15	0.05	0.82	1.13	6.20	239.65	23.08
	Ampicillin(1/4 MIC) +Extract (1/2 MIC)	-0.10	-0.22	0.78	1.02	1.09	6.45	239.14	23.59
	Ampicillin(1/2 MIC) +Extract (1/4 MIC)	-0.06	-0.08	-0.21	0.81	1.50	6.00	220.98	41.75
	Ampi/sul(1/8 MIC) +Extract(1/2 MIC)	-0.09	-0.05	-0.51	1.59	2.08	5.32	217.79	44.94
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	-0.14	-0.09	-0.21	-0.65	1.15	7.06	194.84	67.89

Table A-4: (continue) Log change viable counts at time point and kinetic parameters in 18 isolates of MRSA.

isolates No.	Antimicrobial agent	Viable log change						AUBKC 0-24	Bacteriolytic Area
		Δ2	Δ4	Δ6	Δ8	Δ10	Δ24		
31	control	0.76	1.40	0.98	1.17	1.29	4.62	289.48	-
	Ampi/sul(1/8 MIC)	0.15	0.54	1.17	1.48	1.89	4.14	272.63	16.85
	Ampi/sul(1/2 MIC)	-0.10	-0.29	-0.19	0.74	1.18	6.81	218.35	71.13
	Extract (1/4 MIC)	0.05	0.79	1.20	1.27	2.09	3.52	270.52	18.96
	Extract(1/2 MIC)	-0.04	0.54	0.70	0.52	2.55	4.44	255.01	34.47
	Ampi/sul(1/8 MIC) +Extract(1/2 MIC)	-0.25	-0.23	0.42	1.10	1.25	6.17	229	60.48
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	-0.29	-0.61	-0.15	-0.06	1.35	6.61	196.95	93.19
32	control	0.08	0.85	1.05	1.10	1.03	6.39	273.56	-
	Ampicillin(1/4 MIC)	0.31	0.69	0.52	1.18	1.10	6.98	256.94	16.62
	Ampicillin(1/2 MIC)	-0.74	-0.06	1.01	1.26	0.95	6.09	244.81	28.75
	Ampi/sul(1/8 MIC)	-0.18	1.23	1.57	1.19	0.80	5.04	259.49	14.07
	Ampi/sul(1/2 MIC)	-0.21	-0.20	-0.49	-0.12	1.03	8.23	205.08	68.48
	Extract (1/4 MIC)	0.58	0.34	1.82	0.78	0.37	5.06	258.23	15.33
	Extract (1/2 MIC)	0.76	0.47	0.78	0.85	0.37	6.10	252.23	21.33
	Ampicillin(1/4 MIC) +Extract (1/2 MIC)	-0.12	-0.07	-0.01	0.38	0.77	8.41	223.18	50.38
	Ampicillin(1/2 MIC) +Extract (1/4 MIC)	-0.07	-0.17	0.55	0.97	0.99	7.28	236.19	37.37
	Ampi/sul(1/8 MIC) +Extract(1/2 MIC)	-0.05	-0.15	1.22	0.99	0.95	6.50	246.42	27.14
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	-0.17	-0.10	-0.28	0.49	1.00	7.33	200.52	73.04
94	control	0.33	0.49	0.94	1.32	0.11	6.56	262.23	-
	Ampi/sul(1/4MIC)	-0.10	-0.18	0.47	0.75	1.34	6.79	236.35	25.98
	Ampi/sul(1/2 MIC)	-0.21	-0.21	0.34	0.55	0.66	6.50	181.79	80.44
	Extract (1/4 MIC)	0.01	0.39	0.44	1.11	0.04	5.86	231.83	30.40
	Extract(1/2 MIC)	0.36	0.40	0.41	0.70	0.71	5.65	233.69	28.54
	Ampi/sul(1/4 MIC) +Extract (1/2 MIC)	-0.18	-0.23	-0.29	0.61	1.08	7.12	212.41	49.82
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	-0.17	-0.28	-0.30	-0.49	1.17	6.07	192.22	70.01
102	control	0.27	1.36	1.04	0.98	1.08	4.64	266.99	-
	Ampi/sul(1/4MIC)	0.15	0.48	0.80	0.96	1.15	5.64	253.22	13.77
	Ampi/sul(1/2 MIC)	-0.22	-0.09	-0.38	0.44	1.80	6.39	198.94	68.05
	Extract (1/4 MIC)	0.16	0.46	0.98	1.02	1.33	4.28	243.69	23.30
	Extract(1/2 MIC)	0.06	0.50	0.34	0.05	1.18	6.24	231.61	35.38
	Ampi/sul(1/4 MIC) +Extract (1/2 MIC)	-0.03	-0.30	-0.42	1.05	1.00	6.89	214.19	52.80
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	-0.10	0.10	-0.25	-0.64	1.59	6.91	200.99	66.00
107	control	0.67	0.59	1.14	0.49	2.81	4.23	278.59	-
	Ampicillin(1/8MIC)	-0.04	0.38	0.51	1.76	0.28	6.16	243.75	34.84
	Ampicillin(1/2 MIC)	-0.13	-0.09	-0.16	-0.64	0.90	8.29	202.01	76.58
	Extract (1/4 MIC)	0.22	0.03	0.71	0.40	1.66	5.88	251.05	27.54
	Extract(1/2 MIC)	0.07	0.26	0.87	0.59	1.49	5.04	238.86	39.73
	Ampicillin(1/8 MIC) +Extract (1/2 MIC)	-0.05	-0.13	-0.17	0.79	0.55	7.03	210.42	83.49
	Ampicillin(1/2 MIC) +Extract(1/4 MIC)	-0.12	-0.16	-0.59	-0.31	1.36	6.86	195.10	83.49

Table A-4: (continue) Log change viable counts at time point and kinetic parameters in 18 isolates of MRSA.

isolates No.	Antimicrobial agent	Viable log change						AUBKC 0-24	Bacteriolytic Area
		Δ2	Δ4	Δ6	Δ8	Δ10	Δ24		
234	control	0.18	0.55	1.72	0.60	2.32	4.49	275.76	-
	Ampicillin(1/8MIC)	0.08	0.53	0.34	1.63	0.75	5.43	248.18	27.58
	Ampicillin(1/2 MIC)	-0.17	-0.20	-0.43	-0.23	1.49	7.68	204.72	71.04
	Extract (1/4 MIC)	0.23	0.25	0.49	1.67	-0.20	6.53	237.15	38.61
	Extract(1/2 MIC)	0.03	0.51	0.32	0.41	0.60	6.98	235.67	39.89
	Ampicillin(1/8 MIC) +Extract (1/2 MIC)	-0.30	-0.40	0.78	0.46	0.53	6.89	210.88	64.88
	Ampicillin(1/2 MIC) +Extract(1/4 MIC)	-0.10	-0.57	-0.10	-0.33	0.72	7.75	189.43	86.33
266	control	0.32	0.58	1.58	0.46	1.73	4.55	265.90	-
	Ampicillin(1/4MIC)	-0.13	0.84	0.81	0.73	0.96	5.73	249.90	16
	Ampicillin(1/2 MIC)	-0.17	-0.20	-0.18	0.67	1.17	6.90	214.51	51.39
	Extract (1/4 MIC)	0.51	0.76	0.95	0.63	0.70	5.47	252.69	13.21
	Extract(1/2 MIC)	0.35	0.61	0.78	0.84	0.32	5.79	248.17	17.73
	Ampicillin(1/4 MIC) +Extract (1/2 MIC)	-0.17	-0.19	-0.44	0.73	1.31	6.67	212.49	53.41
	Ampicillin(1/2 MIC) +Extract(1/4 MIC)	-0.16	-0.42	-0.29	-0.14	1.35	7.50	199.00	66.90
268	control	0.55	0.47	1.80	0.69	0.70	5.42	264.49	-
	Ampicillin(1/4MIC)	-0.14	-0.08	0.78	1.61	0.73	4.86	232.16	32.33
	Ampicillin(1/2 MIC)	-0.15	-0.11	-0.20	0.25	1.65	6.74	218.06	46.43
	Extract (1/4 MIC)	0.37	0.25	0.53	1.55	0.28	5.38	239.16	25.33
	Extract(1/2 MIC)	0.23	0.26	0.63	0.53	0.34	7.34	233.17	31.32
	Ampicillin(1/4 MIC) +Extract (1/2 MIC)	-0.06	-0.20	-0.32	1.02	0.54	7.33	210.79	53.70
	Ampicillin(1/2 MIC) +Extract(1/4 MIC)	-0.13	-0.30	-0.44	0.00	0.81	7.07	195.43	69.06
279	control	0.35	0.77	1.15	0.92	1.33	4.88	269.18	-
	Ampi/sul(1/4MIC)	-0.11	-0.18	0.87	0.25	1.41	6.19	229.91	39.27
	Ampi/sul(1/2 MIC)	-0.14	-0.29	-0.19	-0.42	1.35	7.17	203.26	65.92
	Extract (1/4 MIC)	0.50	0.76	0.32	0.73	0.27	5.75	240.63	28.55
	Extract(1/2 MIC)	0.32	0.21	-0.32	0.34	0.78	5.84	236.29	32.89
	Ampi/sul(1/4 MIC) +Extract (1/2 MIC)	-0.08	-0.29	-0.38	1.1	1.06	5.74	211.31	57.87
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	-0.20	-0.18	-0.23	-0.48	1.65	6.96	202.56	66.62
384	control	0.50	0.66	1.63	0.73	0.84	6.12	271.78	-
	Ampicillin(1/4MIC)	-0.14	0.57	0.76	1.33	0.73	6.10	252.57	19.21
	Ampicillin(1/2 MIC)	-0.19	-0.38	-0.30	-0.33	1.43	6.83	201.84	69.94
	Extract (1/4 MIC)	0.44	0.31	0.86	0.77	0.28	5.85	241.69	30.09
	Extract(1/2 MIC)	0.29	0.22	0.69	0.27	0.67	5.91	234.01	37.67
	Ampicillin(1/4 MIC) +Extract (1/2 MIC)	-0.27	-0.12	0.75	0.32	0.68	5.93	213.39	58.39
	Ampicillin(1/2 MIC) +Extract(1/4 MIC)	-0.36	-0.25	-0.23	-0.48	1.55	7.21	202.46	69.32

Table A-4: (continue) Log change viable counts at time point and kinetic parameters in 18 isolates of MRSA.

isolates No.	Antimicrobial agent	Viable log change						AUBKC 0-24	Bacteriolytic Area
		Δ2	Δ4	Δ6	Δ8	Δ10	Δ24		
466	control	0.07	1.37	0.88	0.64	1.01	5.36	291.85	-
	Ampicillin(1/8MIC)	-0.13	0.34	0.46	-0.67	0.83	6.15	227.62	63.38
	Ampicillin(1/2 MIC)	-0.05	-0.22	-0.37	-0.46	1.67	6.16	197.69	94.16
	Extract (1/4 MIC)	0.26	0.25	0.20	0.53	-1.50	8.68	219.86	71.99
	Extract(1/2 MIC)	0.22	0.18	0.24	0.25	0.78	6.18	225.73	66.12
	Ampicillin(1/8 MIC) +Extract (1/2 MIC)	-0.08	-0.12	-0.70	0.51	0.51	7.46	208.17	83.68
	Ampicillin(1/2 MIC) +Extract(1/4 MIC)	-0.19	-0.08	-0.32	-0.47	1.02	6.90	195.64	96.21
777	control	0.09	0.98	0.90	1.57	0.53	6.01	267.66	-
	Ampicillin(1/8 MIC)	0.06	1.02	0.84	1.07	0.99	6.25	267.47	0.19
	Ampicillin(1/2 MIC)	-0.02	-0.05	-0.14	0.37	0.92	8.06	224.02	43.64
	Ampi/sul(1/4 MIC)	-0.09	-0.11	-0.08	0.19	1.01	8.27	224.84	42.82
	Ampi/sul(1/2 MIC)	-0.05	-0.29	-0.17	-0.76	1.29	7.99	205.25	62.41
	Extract (1/4 MIC)	-0.08	0.88	0.25	1.18	0.89	7.22	257.74	9.92
	Extract (1/2 MIC)	0.08	0.13	0.76	1.11	0.87	7.31	254.83	12.92
	Ampicillin(1/8 MIC) +Extract (1/2 MIC)	-0.03	0.20	-0.14	0.89	0.93	7.56	232.61	35.05
	Ampicillin(1/2 MIC) +Extract (1/4 MIC)	-0.07	-0.03	0.22	0.92	1.06	7.12	235.72	31.94
	Ampi/sul(1/4 MIC) +Extract(1/2 MIC)	-0.12	-0.03	0.25	0.68	0.89	7.40	231.62	36.04
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	-0.04	-0.41	-0.12	-0.60	1.67	7.72	211.88	55.68
786	control	0.27	1.46	0.87	1.11	0.37	5.09	261.05	-
	Ampi/sul(1/4MIC)	-0.27	-0.32	0.88	1.57	0.48	5.12	240.56	20.49
	Ampi/sul(1/2 MIC)	-0.19	-0.06	-0.28	0.62	1.15	6.93	195.47	65.58
	Extract (1/4 MIC)	0.24	0.54	0.37	-0.65	0.28	7.57	237.09	23.96
	Extract(1/2 MIC)	0.14	0.51	0.47	0.52	0.46	6.98	230.44	30.61
	Ampi/sul(1/4 MIC) +Extract (1/2 MIC)	-0.08	-0.07	-0.19	0.68	1.16	6.72	221.24	39.81
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	-0.08	-0.21	-0.24	-0.51	1.88	6.44	197.14	63.91
1028	control	0.35	0.16	0.93	0.72	0.38	7.70	257.16	-
	Ampi/sul(1/4MIC)	-0.07	-0.08	0.18	1.10	1.44	7.06	227.45	29.71
	Ampi/sul(1/2 MIC)	-0.09	-0.33	-0.54	-0.12	0.59	7.62	186.57	70.59
	Extract (1/4 MIC)	0.43	0.03	0.09	0.52	0.53	7.53	236.29	20.87
	Extract(1/2 MIC)	0.45	0.11	0.10	0.55	1.00	7.18	243.73	13.43
	Ampi/sul(1/4 MIC) +Extract (1/2 MIC)	-0.07	-0.39	-0.44	1.49	0.36	7.18	208.64	48.52
	Ampi/sul(1/2 MIC) +Extract(1/4 MIC)	-0.16	0.23	-0.35	-0.44	0.93	7.88	169.39	87.77

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ↑

Ampicillin →

Figure A-1: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.3 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ↑

Ampicillin →

Figure A-2: The additive result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.9 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ⟶

⟶ Ampicillin

Figure A-3: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.17 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ⟶

⟶ Ampicillin

Figure A-4: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.19 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ⟶

⟶ Ampicillin

Figure: A-5: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.20 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ⟶

⟶ Ampicillin

Figure A-6: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.23 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ↑

→ Ampicillin

Figure A-7: The additve result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.31 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ↑

→ Ampicillin

Figure A-8: partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.32 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* →

→ Ampicillin

Figure A-9: The additive result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.34 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* →

→ Ampicillin

Figure A-10: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.38 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ⟶

⟶ Ampicillin

Figure A-11: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.94 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ⟶

⟶ Ampicillin

Figure A-12: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.102 Shadow zone : visible microorganism growth, white zone : no microorganism growth

Extract of *H.odorata*								
250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Ampicillin

Figure A-13: The synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.107 Shadow zone : visible microorganism growth, white zone : no microorganism growth

Extract of *H.odorata*								
250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Ampicillin

Figure A-14: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.152 Shadow zone : visible microorganism growth, white zone : no microorganism growth

Extract of *H.odorata*								
250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Ampicillin

Figure A-15: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.200 Shadow zone : visible microorganism growth, white zone : no microorganism growth

Extract of *H.odorata*								
250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Ampicillin

Figure A-16: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.216 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ↑

Ampicillin →

Figure A-17: The additive result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.240 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ↑

Ampicillin →

Figure A-18: The partial synergism result (checkerboard) of alcoholic extract of *H .odorata* ROXB plus ampicillin against MRSA isolate no.234 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ↑

Ampicillin →

Figure A-19: The partial synergism result (checkerboard) of alcoholic extract of *H .odorata* ROXB plus ampicillin against MRSA isolate no.241 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ↑

Ampicillin →

Figure A-20: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.266 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ↑

→ Ampicillin

Figure A-21: The partial synergism result (checkerboard) of alcoholic extract of *H .odorata* ROXB plus ampicillin against MRSA isolate no.268 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ↑

→ Ampicillin

Figure A-22: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.269 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ↑

Ampicillin →

Figure A-23: The additive result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.279 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ↑

Ampicillin →

Figure A-24: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.384 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ↑

Ampicillin →

Figure A-25: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.466 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ↑

Ampicillin →

Figure A-26: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.643 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ↑

Ampicillin →

Figure A-27: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.777 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ↑

Ampicillin →

Figure A-28: The additive result (checkerboard) of alcoholic extract of *H .odorata* ROXB plus ampicillin against MRSA isolate no.786 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ↑

Ampicillin →

Figure A-29: The additive result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.F9 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/2	250/4	250/8	250/16	250/32	250/64	250/128	250/256
125	125/2	125/4	125/8	125/16	125/32	125/64	125/128	125/256
62.5	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128	62.5/256
31.2	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128	31.2/256
15.6	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128	15.6/256
7.8	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128	7.8/256
3.9	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128	3.9/256
1.95	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128	1.95/256
0	2	4	8	16	32	64	128	256

Extract of *H.odorata* ↑

Ampicillin →

Figure A-30 : The synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin against MRSA isolate no.1028 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ↑

→ Ampicillin/sulbactam

Figure A-31: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.3 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ↑

→ Ampicillin/sulbactam

Figure A-32: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.9 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ↑

Ampicillin/sulbactam →

Figure A-33: The additive result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.17 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ↑

Ampicillin/sulbactam →

Figure A-34: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.19 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata*

Ampicillin/sulbactam

Figure A-35: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.20 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata*

Ampicillin/sulbactam

Figure A-36: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.23 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ⟶

⟶ Ampicillin/sulbactam

Figure A-37: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.31 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ⟶

⟶ Ampicillin/sulbactam

Figure A-38: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.32 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata*

Ampicillin/sulbactam

Figure A-39: The additive result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.34 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata*

Ampicillin/sulbactam

Figure A-40: The additive result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.38 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ↑

Ampicillin/sulbactam →

Figure A-41: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.94 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ↑

Ampicillin/sulbactam →

Figure A-42: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.102 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ↑

Ampicillin/sulbactam →

Figure A-43: The additive result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.107 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ↑

Ampicillin/sulbactam →

Figure A-44: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.152 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ↑

Ampicillin/sulbactam →

Figure A-45: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.200 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ↑

Ampicillin/sulbactam →

Figure A-46: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.216 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ↑

Ampicillin/sulbactam →

Figure A-47: The additive result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.234 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ↑

Ampicillin/sulbactam →

Figure A-48: The additive result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.240 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ↑

Ampicillin/sulbactam →

Figure A-49: The additive result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.241 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ↑

Ampicillin/sulbactam →

Figure A-50: The additive result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.266 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ↑

Ampicillin/sulbactam →

Figure A-51: The additive result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.268 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ↑

Ampicillin/sulbactam →

Figure A-52: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.269 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ⟶

⟶ Ampicillin/sulbactam

Figure A-53: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.279 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ⟶

⟶ Ampicillin/sulbactam

Figure A-54: The additive result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.384 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ↑

Ampicillin/sulbactam →

Figure A-55: The additive result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.466 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ↑

Ampicillin/sulbactam →

Figure A-56: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.643 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ↑

Ampicillin/sulbactam →

Figure A-57: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.777 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ↑

Ampicillin/sulbactam →

Figure A-58: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.786 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ⟶

⟶ Ampicillin/sulbactam

Figure A-59: The partial synergism result (checkerboard) of alcoholic extract of *H. odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.F9 Shadow zone : visible microorganism growth, white zone : no microorganism growth

250	250/1	250/2	250/4	250/8	250/16	250/32	250/64	250/128
125	125/1	125/2	125/4	125/8	125/16	125/32	125/64	125/128
62.5	62.5/1	62.5/2	62.5/4	62.5/8	62.5/16	62.5/32	62.5/64	62.5/128
31.2	31.2/1	31.2/2	31.2/4	31.2/8	31.2/16	31.2/32	31.2/64	31.2/128
15.6	15.6/1	15.6/2	15.6/4	15.6/8	15.6/16	15.6/32	15.6/64	15.6/128
7.8	7.8/1	7.8/2	7.8/4	7.8/8	7.8/16	7.8/32	7.8/64	7.8/128
3.9	3.9/1	3.9/2	3.9/4	3.9/8	3.9/16	3.9/32	3.9/64	3.9/128
1.95	1.95/1	1.95/2	1.95/4	1.95/8	1.95/16	1.95/32	1.95/64	1.95/128
0	1	2	4	8	16	32	64	128

Extract of *H.odorata* ⟶

⟶ Ampicillin/sulbactam

Figure A-60: The partial synergism result (checkerboard) of alcoholic extract of *H .odorata* ROXB plus ampicillin /sulbactam against MRSA isolate no.1028 Shadow zone : visible microorganism growth, white zone : no microorganism growth

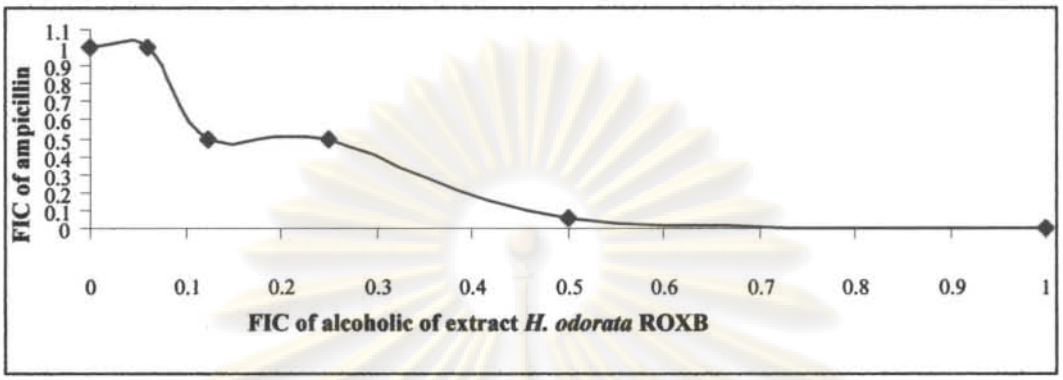

Figure A-61: The isobologram of alcoholic extract (*H. odorata* ROXB) plus ampicillin combinations against 1 isolates of MRSA no. 466

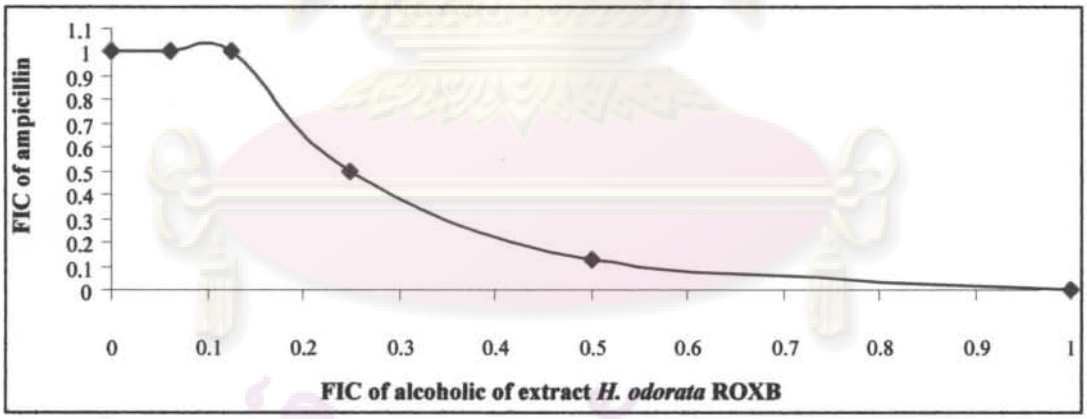

Figure A-62: The isobologram of the alcoholic extract (*H. odorata* ROXB) plus ampicillin combinations against 2 isolates of MRSA no. 17 and 777

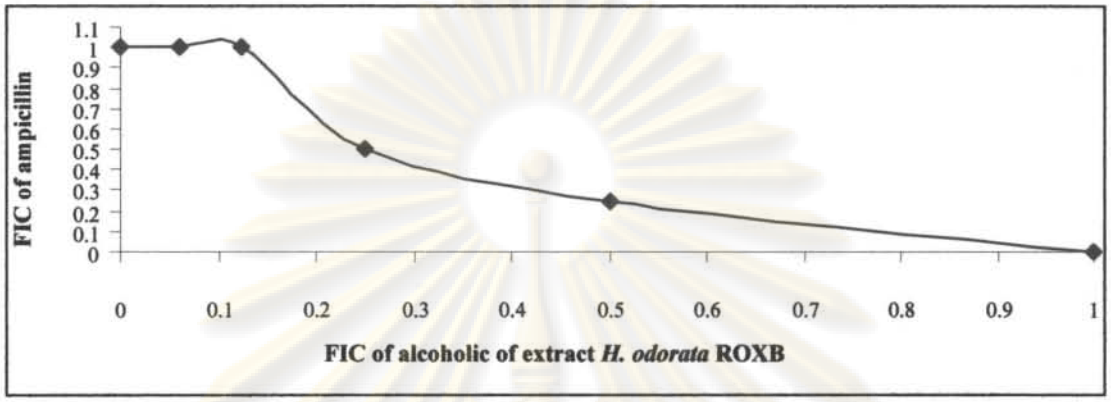

Figure A-63: The isobologram of the alcoholic extract (*H. odorata* ROXB) plus ampicillin combinations against 5 isolates of MRSA no. 20, 23, 32, 266, and 384

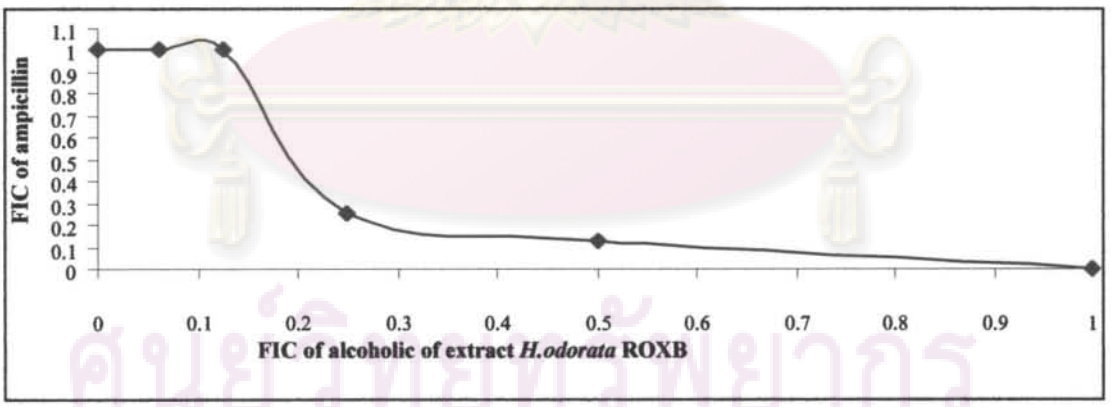

Figure A-64: The isobologram of the alcoholic extract (*H. odorata* ROXB) plus ampicillin combinations against 1 isolates of MRSA no.107

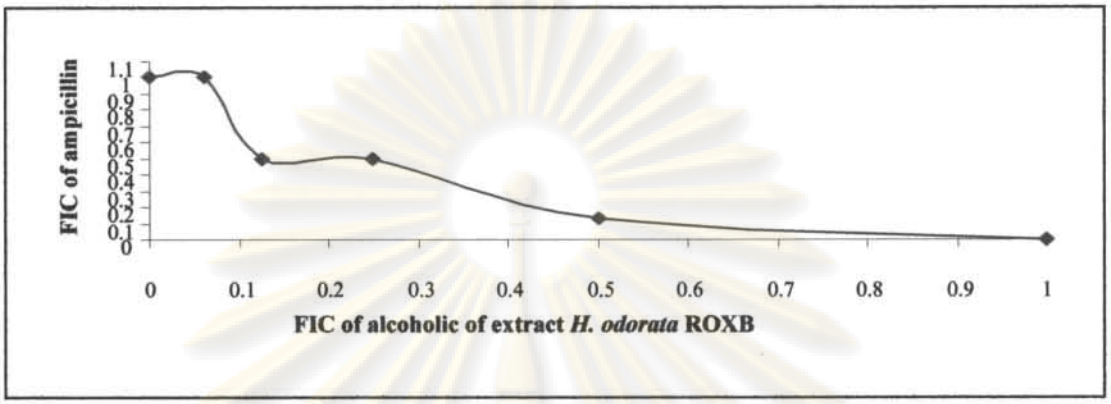

Figure A-65: The isobologram of the alcoholic extract (*H. odorata* ROXB) plus ampicillin combinations against 1 isolate of MRSA no.234

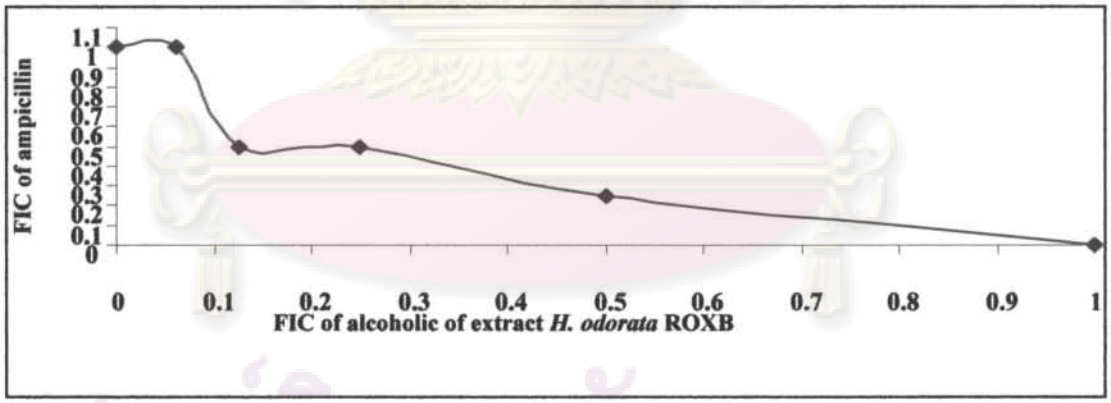

Figure A-66: The isobologram of the alcoholic extract (*H. odorata* ROXB) plus ampicillin combinations against 1 isolate of MRSA no.268

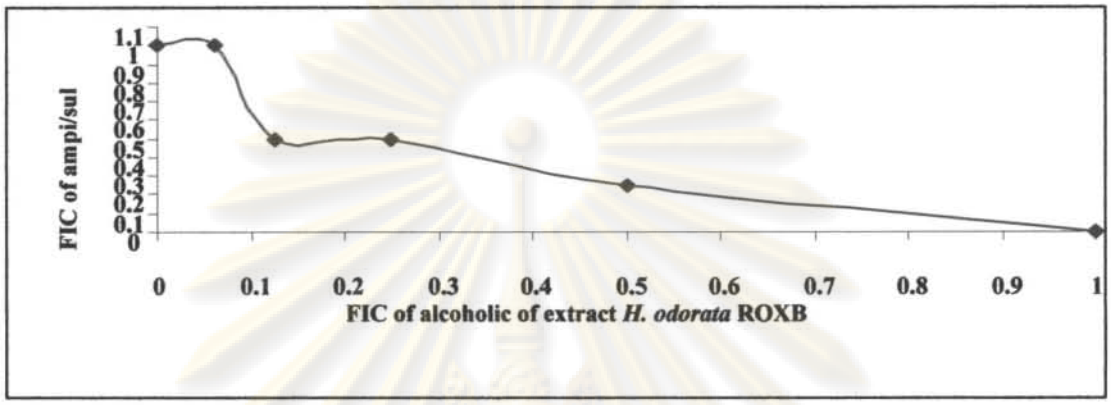

Figure A-67: The isobologram of the alcoholic extract (*H. odorata* ROXB) plus ampicillin/sulbactam combinations against 4 isolates of MRSA no.3, 102, 777, and 786

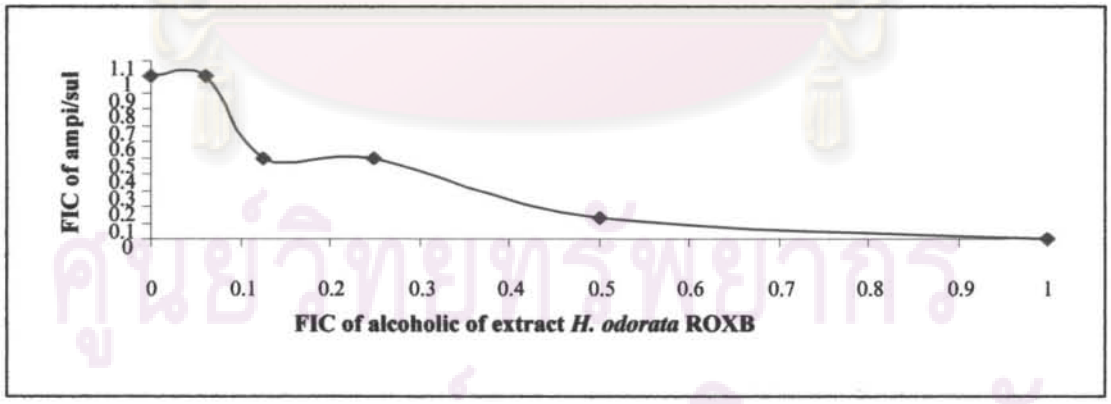

Figure A-68: The isobologram of the alcoholic extract (*H.odorata* ROXB) plus ampicillin/sulbactam combinations against 1 isolates of MRSA no.20

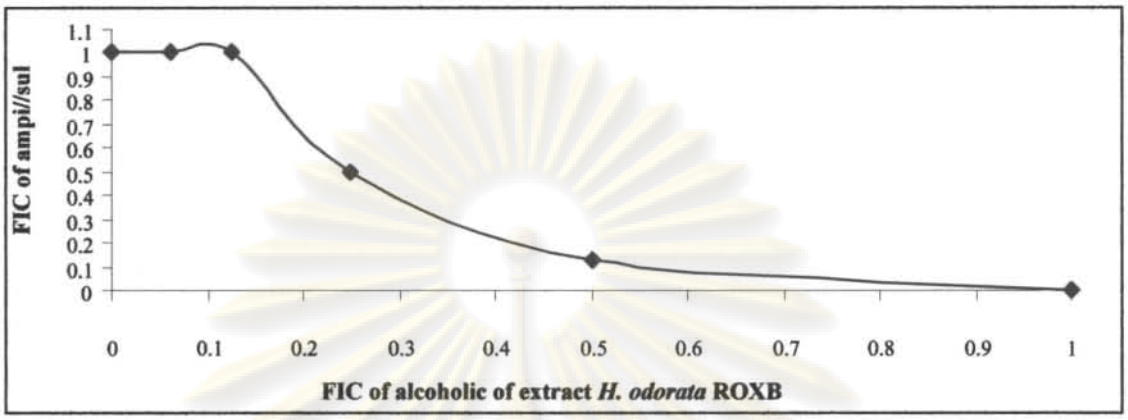

Figure A-69: The isobologram of the alcoholic extract (*H. odorata* ROXB) plus ampicillin/sulbactam combinations against 3 isolates of MRSA no.23, 31, and 32

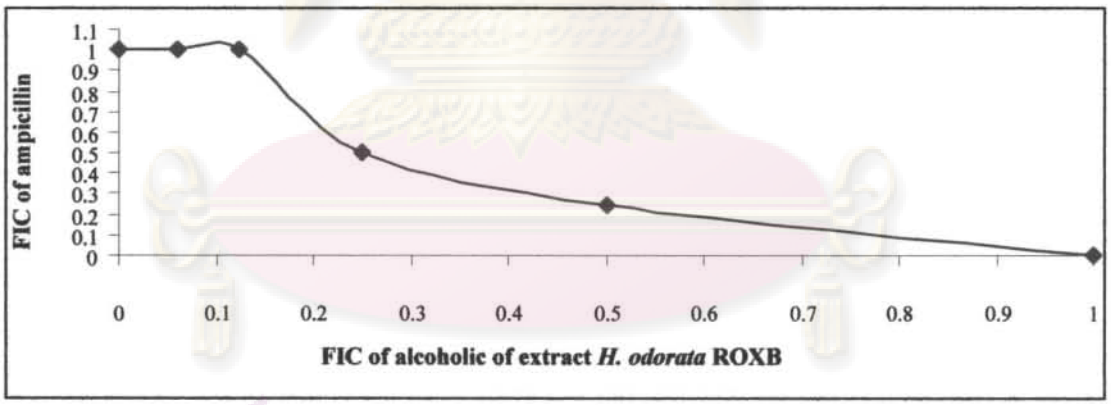

Figure A-70: The isobologram of the alcoholic extract (*H. odorata* ROXB) plus ampicillin/sulbactam combinations against 3 isolates of MRSA no.94, 279, and 1028

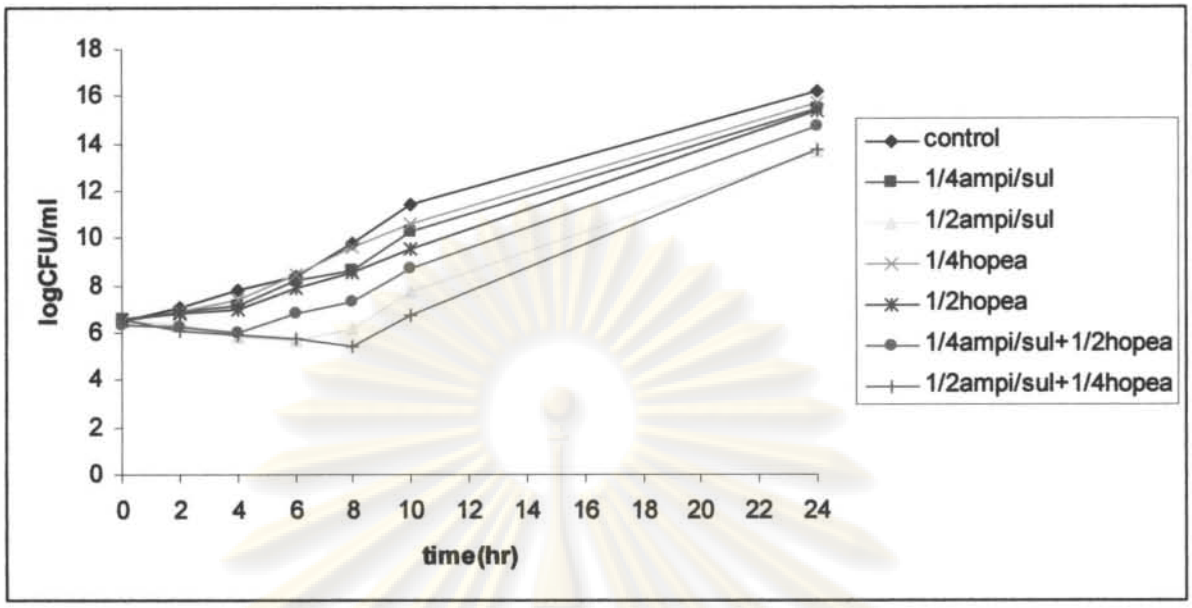

Figure A -71: Time kill curves showing the antibacterial activity of the combination of ¼ MIC Ampicillin plus ½ MIC alcoholic extract of *H. odorata* ROXB. against MRSA isolate no. 3

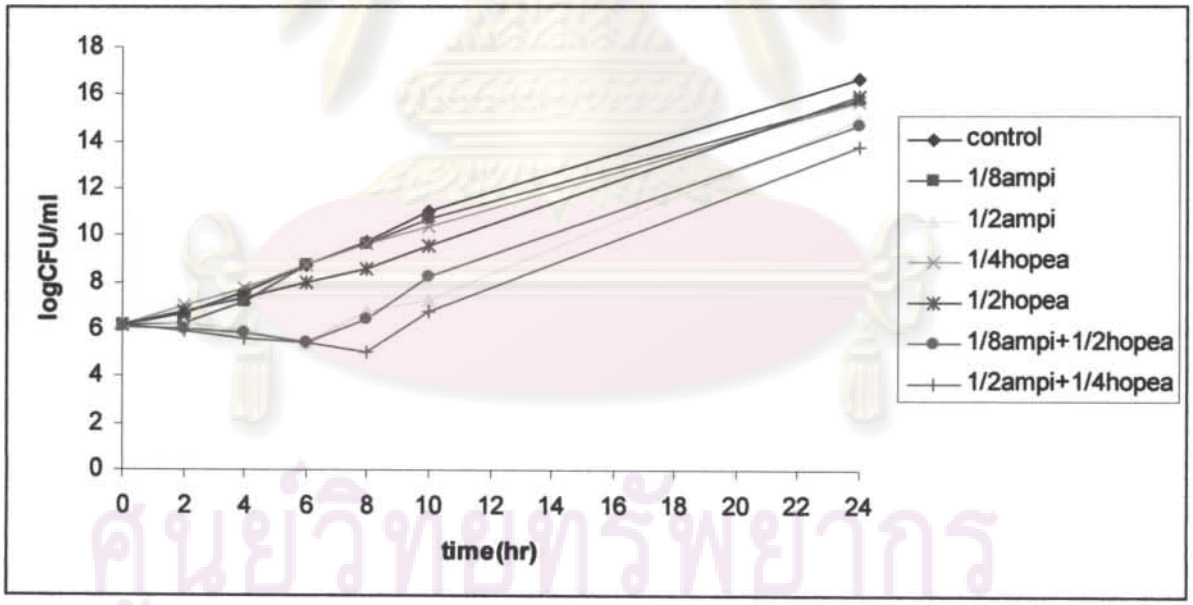

Figure A -72: Time kill curves showing the antibacterial activity of the combination of ¼ MIC ampicillin/sulbactam plus ½ MIC alcoholic extract of *H. odorata* ROXB against MRSA isolate no. 17

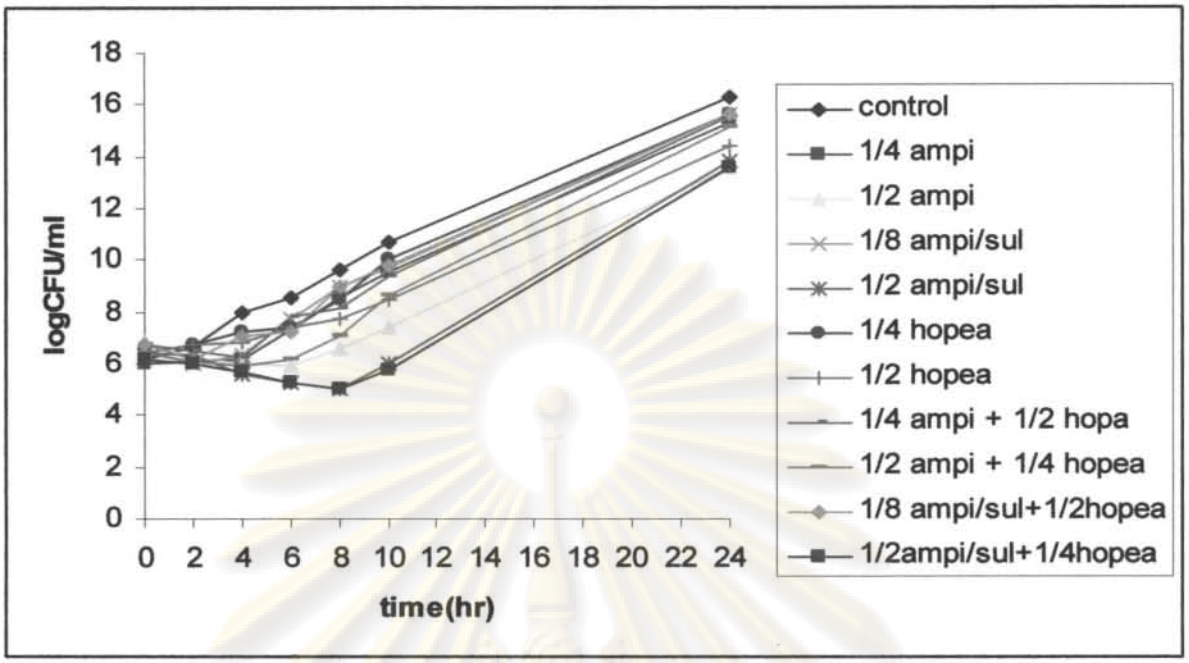

Figure A -73: Time kill curves showing the antibacterial activity of the combination of 1/4 MIC ampicillin plus 1/2 MIC alcoholic extract of *H. odorata* ROXB. and the combination of 1/8 MIC ampicillin/sulbactam plus 1/2 MIC alcoholic extract of *H. odorata* ROXB. against MRSAisolate no. 20

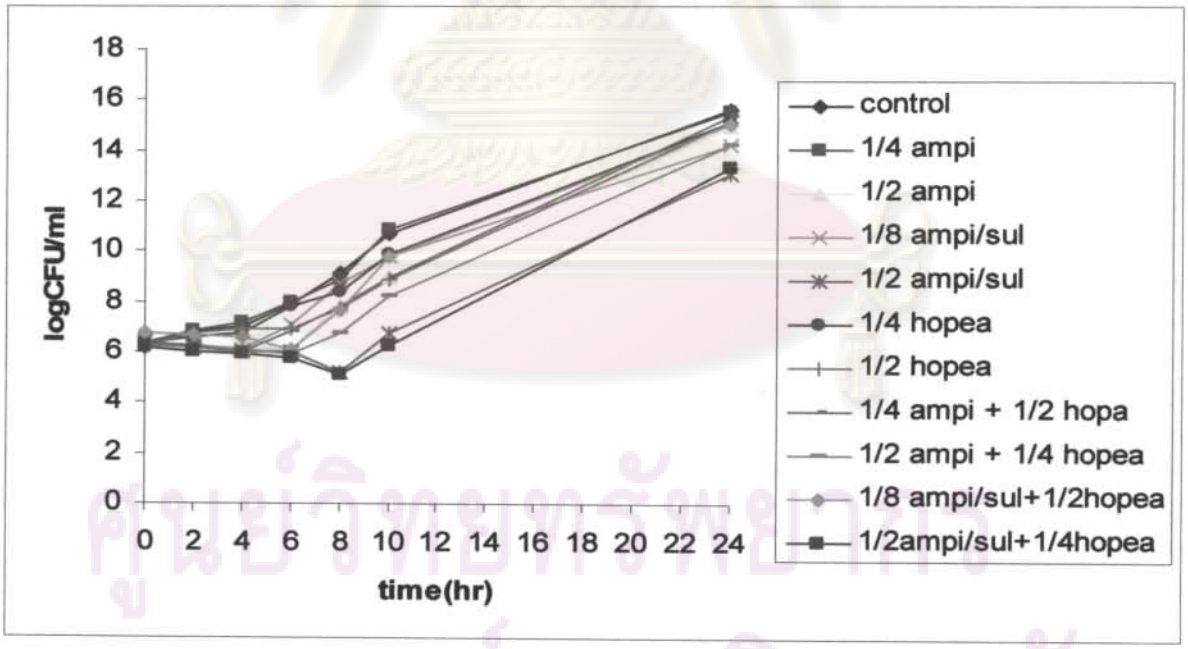

Figure A -74: Time kill curves showing the antibacterial activity of the combination of 1/4 MIC ampicillin plus 1/2 MIC alcoholic extract of *H. odorata* ROXB and the combination of 1/8 MIC ampicillin/sulbactam plus 1/2MIC alcoholic extract of *H. odorata* ROXB. against MRSA isolate no. 23

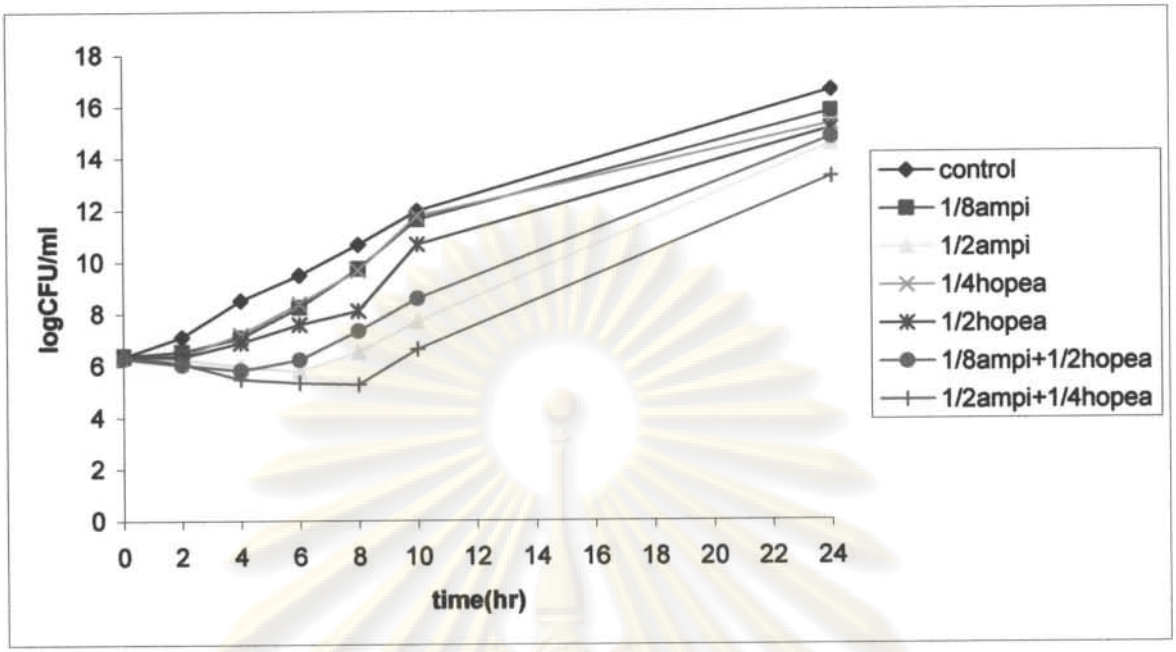

Figure A -75: Time kill curves showing the antibacterial activity of the combination of 1/8 MIC ampicillin plus 1/2 MIC alcoholic extract of *H. odorata* ROXB. against MRSA isolate no. 31

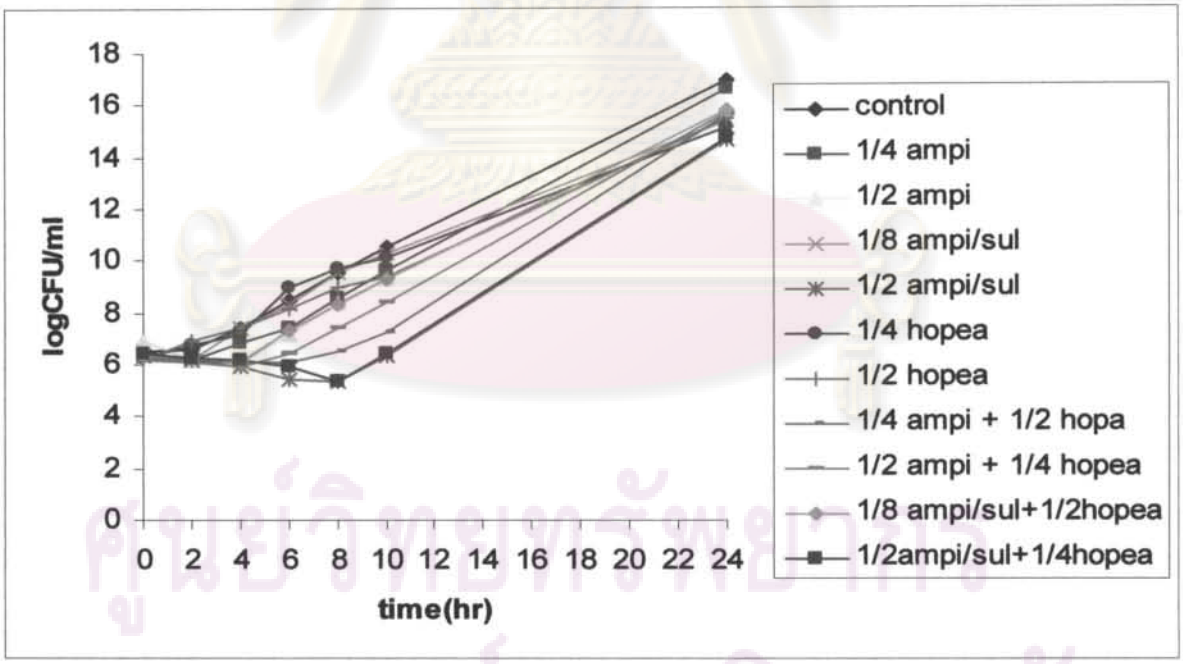

Figure A -76: Time kill curves showing the antibacterial activity of the combination of 1/4 MIC ampicillin plus 1/2 MIC alcoholic extract of *H. odorata* ROXB. and the combination of 1/8 MIC ampicillin/sulbactam plus 1/2 MIC alcoholic extract of *H. odorata* ROXB. against MRSA isolate no. 32

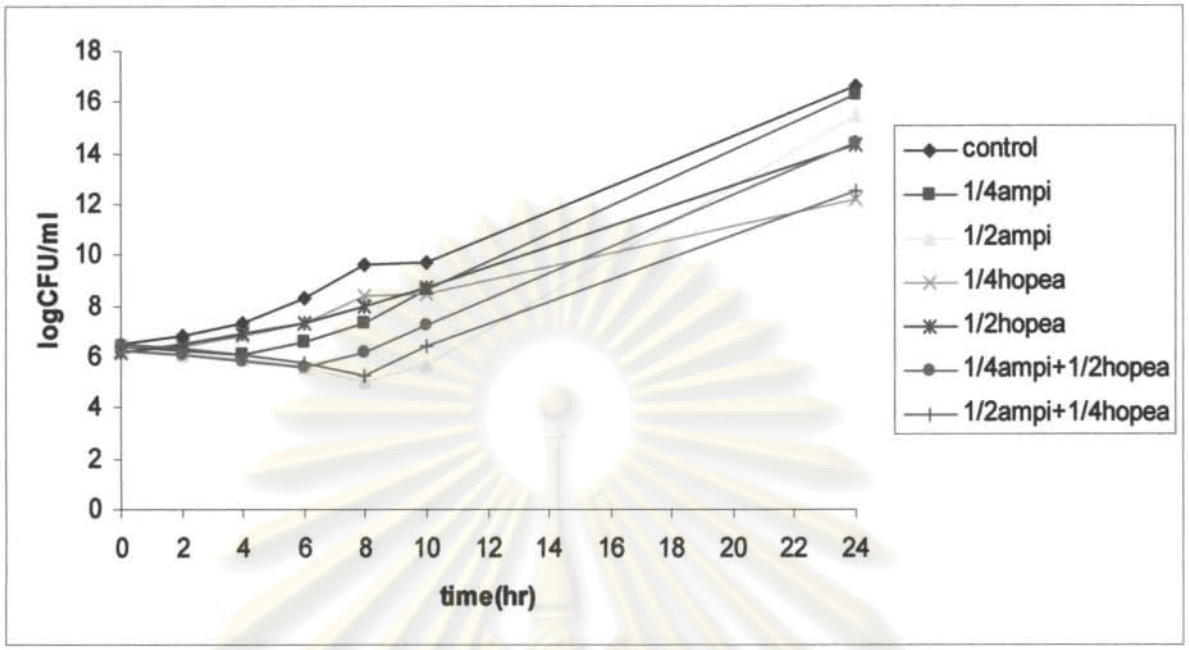

Figure A -77: Time kill curves showing the antibacterial activity of the combination of ¼ MIC ampicillin plus alcoholic extract of ½ MIC *H. odorata* ROXB against MRSA isolate no. 94

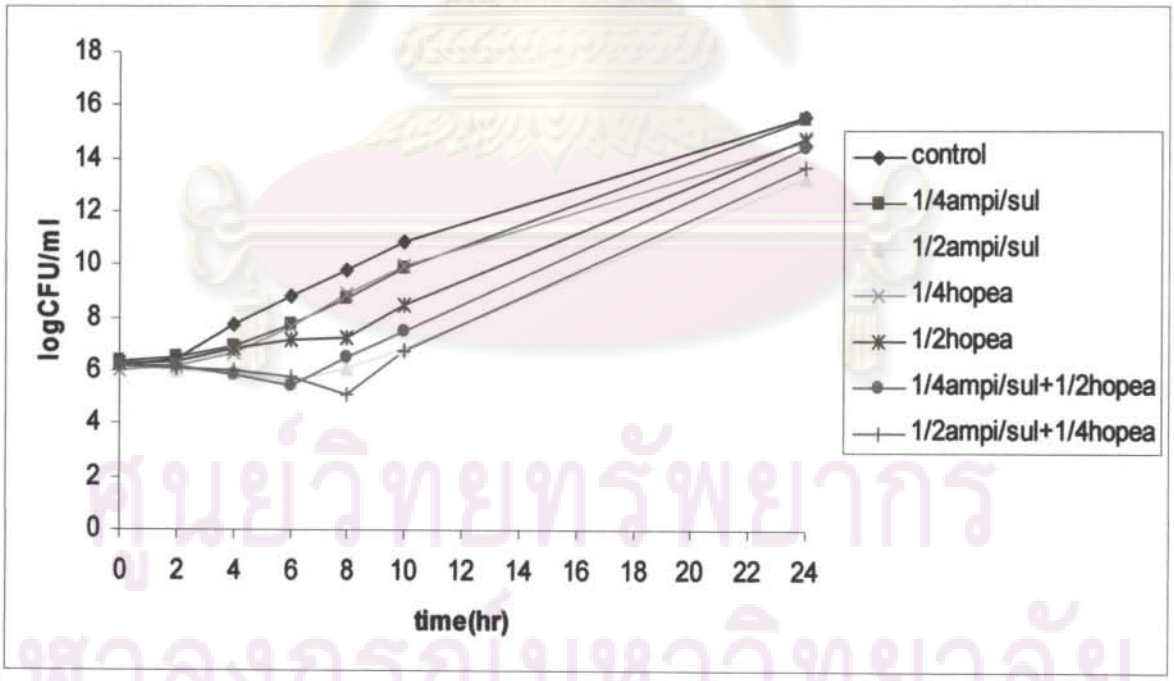

Figure A -78: Time kill curves showing the antibacterial activity of the combination of ¼ MIC ampicillin/sulbactam plus ½ MIC alcoholic extract of *H. odorata* ROXB. against MRSA isolate no. 102

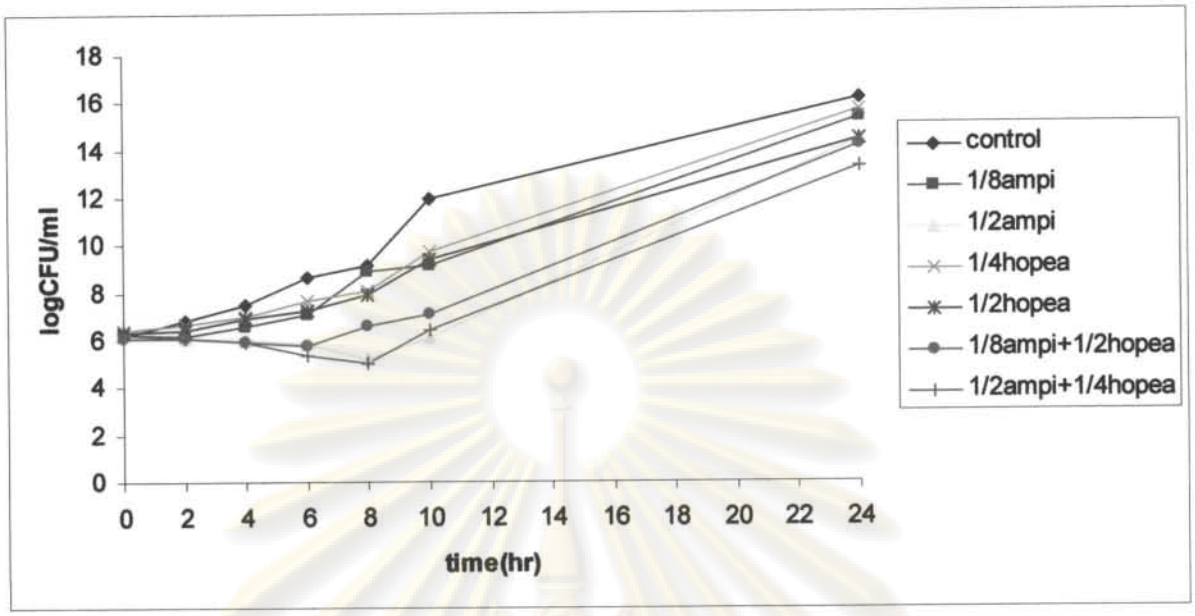

Figure A -79: Time kill curves showing the antibacterial activity of the combination of 1/8 MIC ampicillin plus 1/2 MIC alcoholic extract of *H. odorata* ROXB. against MRSA isolate no.107

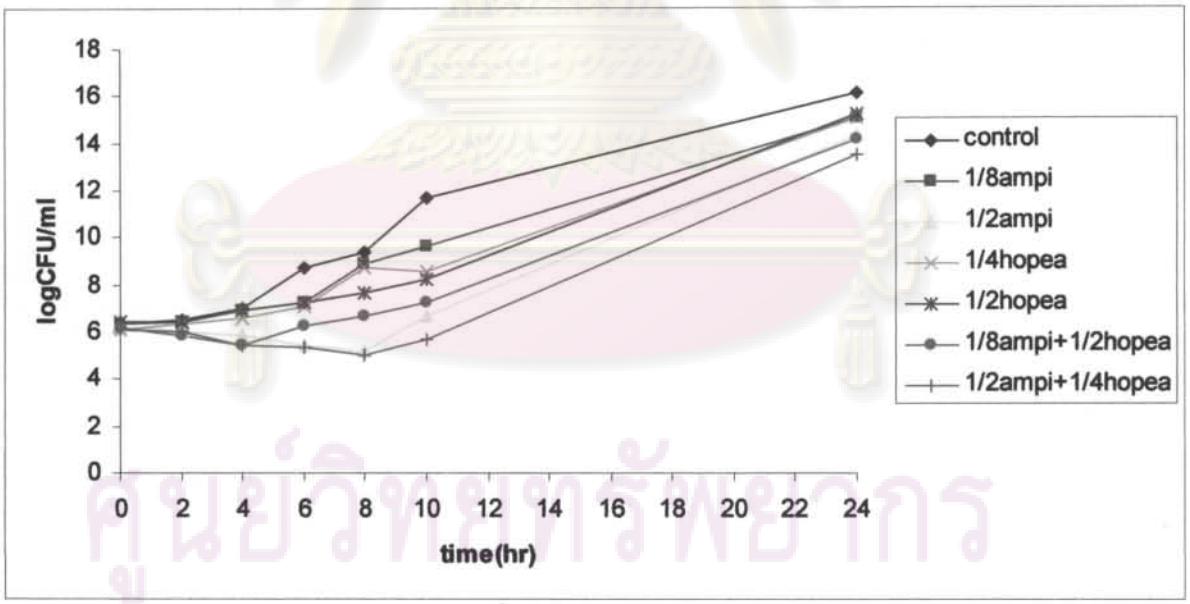

Figure A -80: Time kill curves showing the antibacterial activity of the combination of 1/8 MIC ampicillin plus 1/2 MIC alcoholic extract of *H. odorata* ROXB. against MRSA isolate no.234

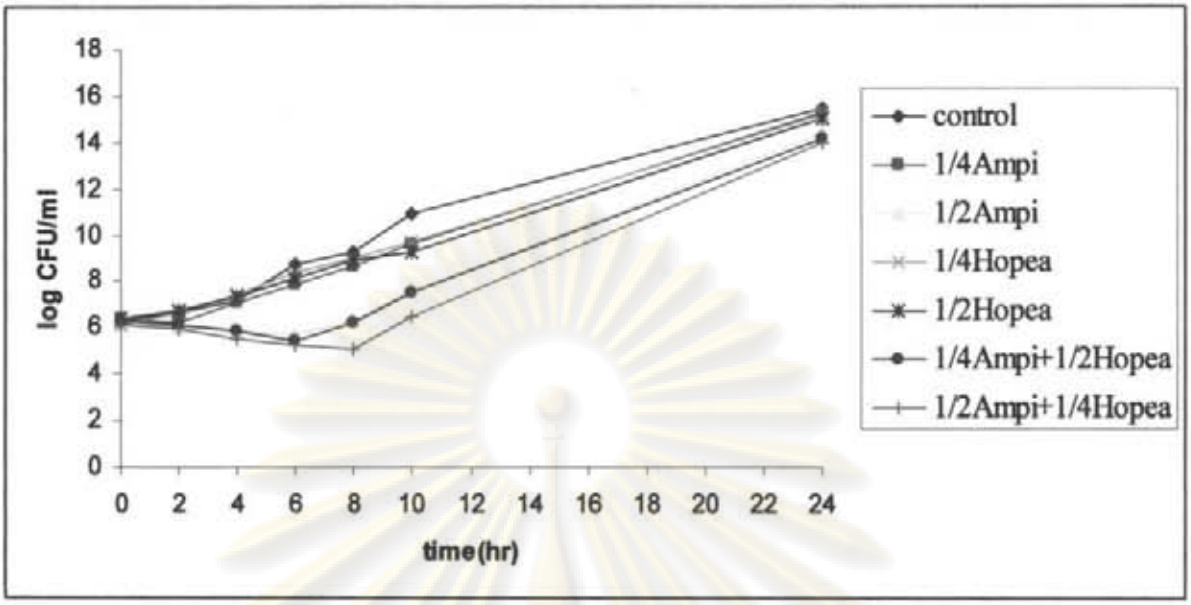

Figure A -81: Time kill curves showing the antibacterial activity of the combination of ¼ MIC ampicillin plus ½ MIC alcoholic extract of *H. odorata* ROXB. against MRSA isolate no.266

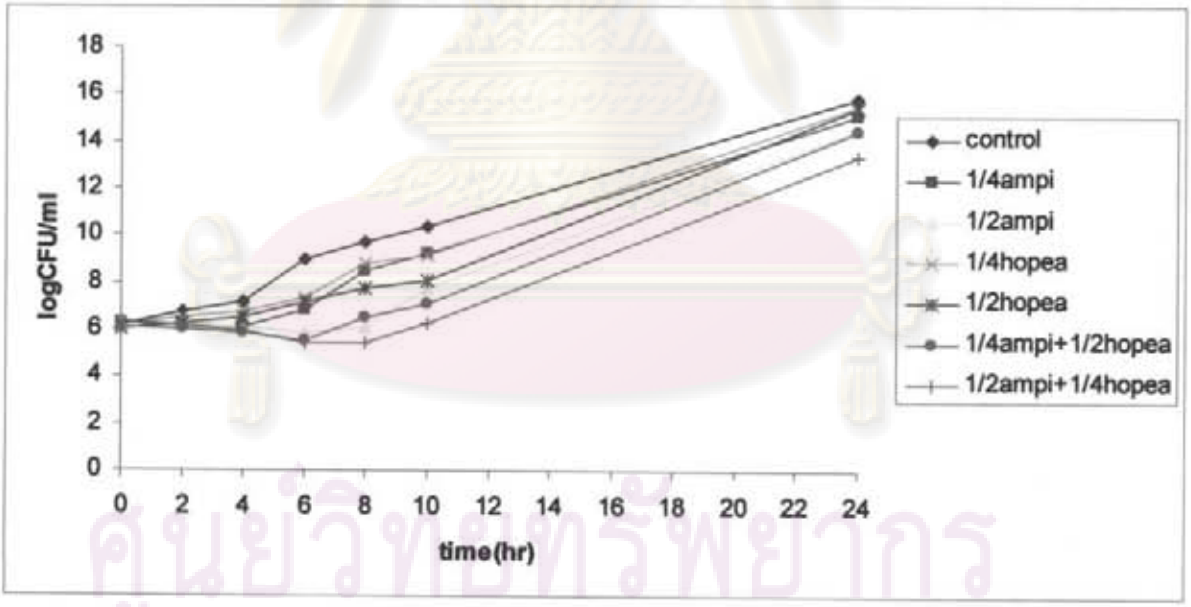

Figure A -82: Time kill curves showing the antibacterial activity of the combination of ¼ MIC ampicillin plus ½ MIC alcoholic extract of *H. odorata* ROXB. against MRSA isolate no.268

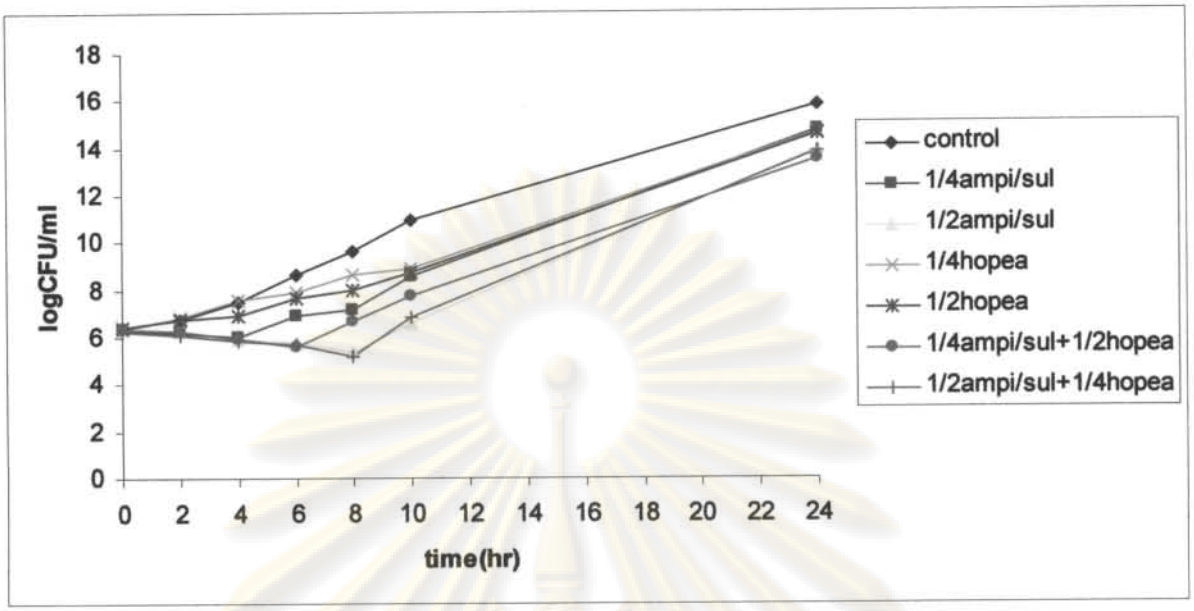

Figure A -83: Time kill curves showing the antibacterial activity of the combination of ¼ MIC ampicillin/sulbactam plus ½ MIC alcoholic extract of *H. odorata* ROXB.against MRSA isolate no.279

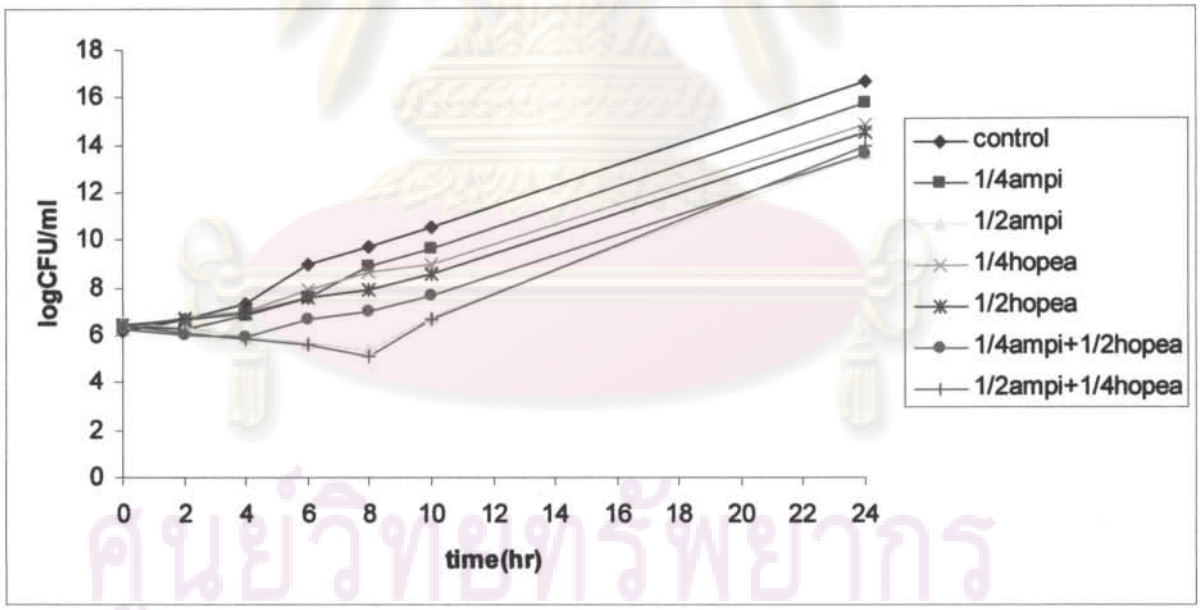

Figure A -84 Time kill curves showing the antibacterial activity of the combination of ¼ MIC ampicillin plus ½ MIC alcoholic extract of *H. odorata* ROXB. against MRSA isolate no.384

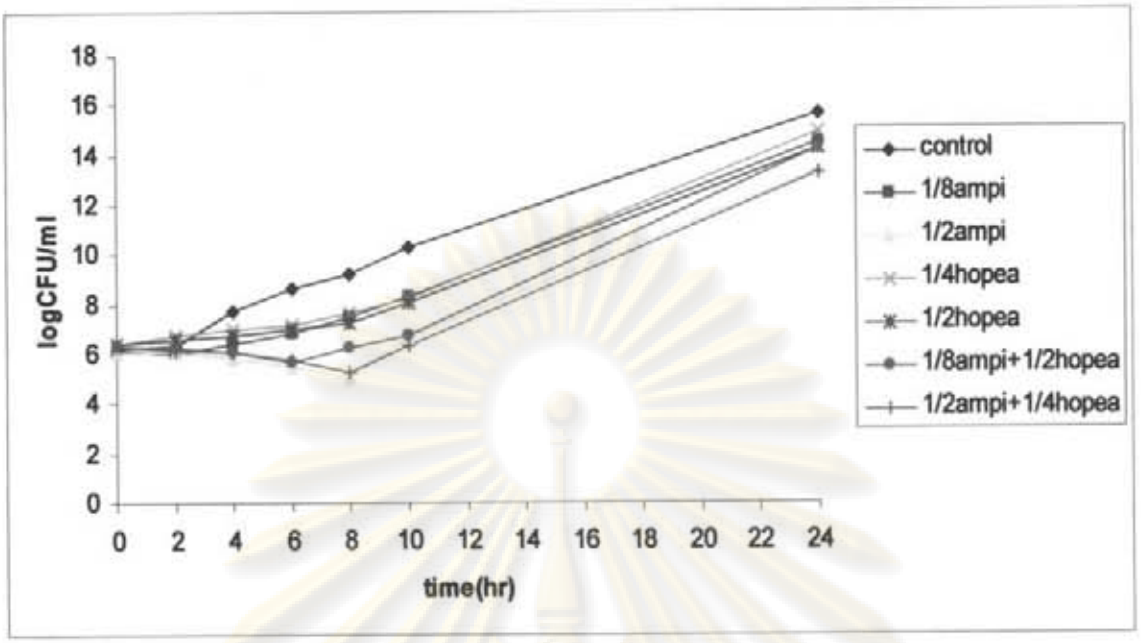

Figure A -85: Time kill curves showing the antibacterial activity of the combination of 1/8 MIC ampicillin plus 1/2 MIC alcoholic extract of *H. odorata* ROXB. against MRSA isolate no.466

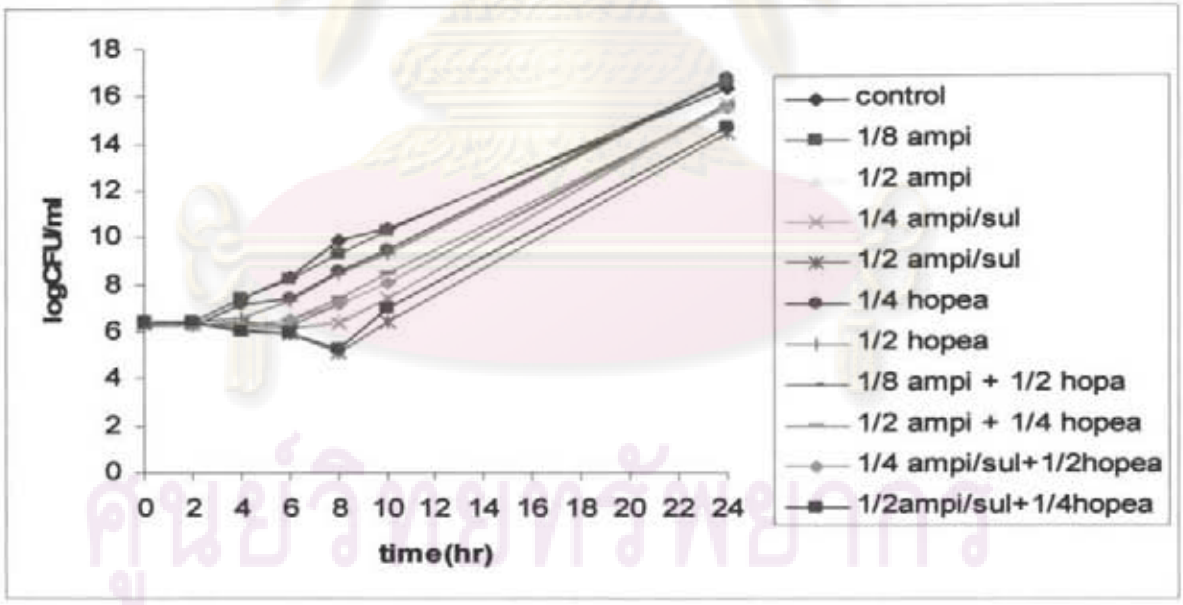

Figure A -86: Time kill curves showing the antibacterial activity of the combination of 1/8 MIC ampicillin plus 1/2 MIC alcoholic extract of *H. odorata* ROXB. and the combination of 1/4 MIC ampicillin/sulbactam plus 1/2 MIC alcoholic extract of *Hopea odorata* ROXB. against MRSA isolate no. 777

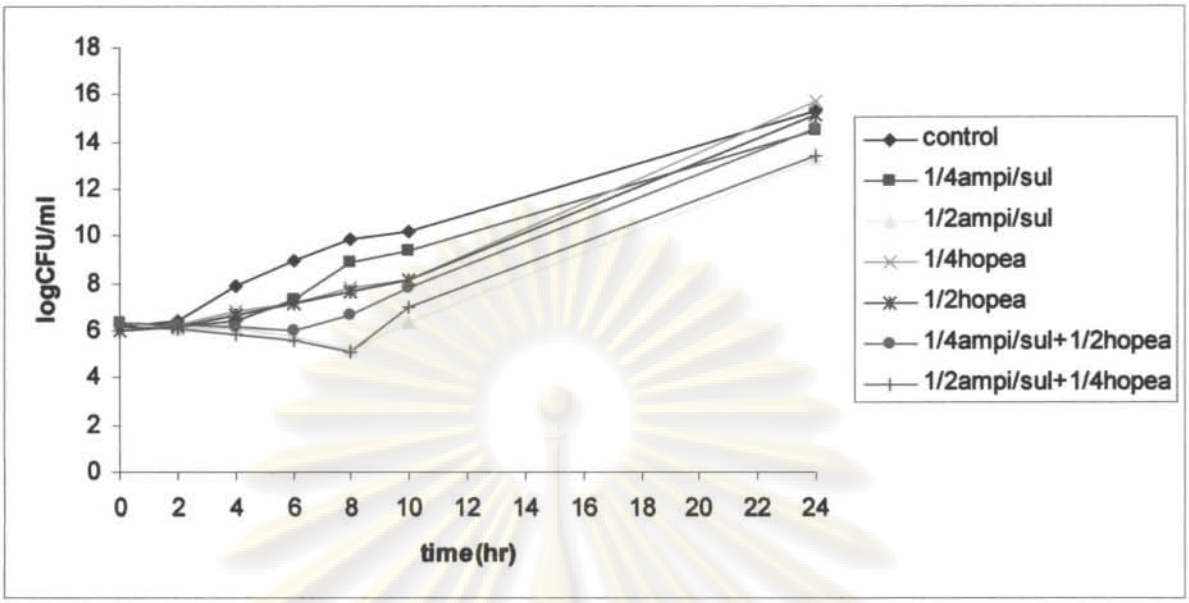

Figure A -87: Time kill curves showing the antibacterial activity of the combination of ¼ MIC ampicillin/sulbactam plus ½ MIC alcoholic extract of *H. odorata* ROXB. against MRSA isolate no.786

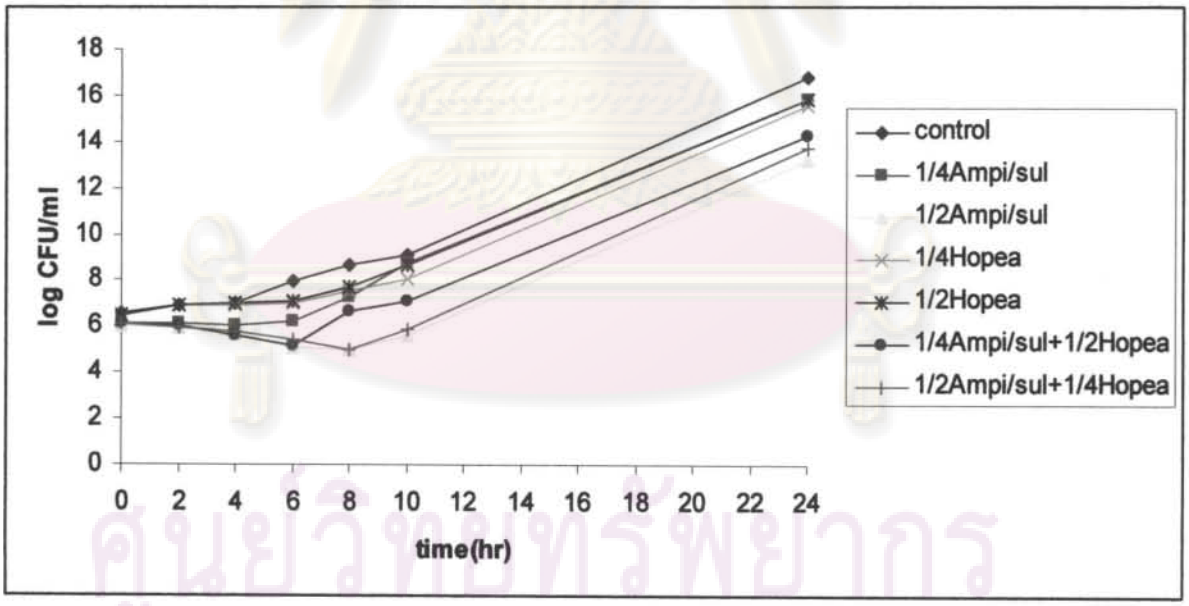

Figure A -88: Time kill curves showing the antibacterial activity of the combination of ¼ MIC ampicillin/sulbactam plus ½ MIC alcoholic extract of *H. odorata* ROXB. against MRSA isolate no1028

BIOGROPHY

My name is Wipasinee Wannasak, I was born on July 30, 1980 in Chiangmai. I have graduated with the bachelor degree in Nursing Science from Chiangmai University since 2002. I have enrolled the master's program in Pharmacology (Inter-Department), Faculty of Graduate school, Chulalongkorn University since June 2004.