

ผลของสารสกัดแอลกอฮอล์จากผลสมอติ่งต่อเชื้อ *Escherichia coli* สายพันธุ์ที่สร้างเอนไซม์
extended spectrum beta-lactamase

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EFFECTS OF *TERMINALIA CITRINA* ROXB. ALCOHOLIC EXTRACT ON EXTENDED
SPECTRUM BETA-LACTAMASE PRODUCING *ESCHERICHIA COLI*

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จุฬามณี เวียงวงศ์ : ผลของสารสกัดแอลกอฮอล์จากผลสมอติ่งต่อเชื้อ *Escherichia coli* สายพันธุ์ที่สร้างเอนไซม์ extended spectrum beta-lactamase. (EFFECTS OF *TERMINALIA CITRINA* ROXB. ALCOHOLIC EXTRACT ON EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING *ESCHERICHIA COLI*)

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เชื้อ *E.coli* สายพันธุ์ที่สร้างเอนไซม์ extended spectrum beta-lactamases (ESBLs) ก่อปัญหาที่สำคัญมากในทางการแพทย์และสาธารณสุขเพราะทำให้เชื้อดื้อยาในกลุ่ม betalactam ได้หลายชนิด และ *E.coli* บางสายพันธุ์ที่ได้รับการถ่ายทอดการดื้อยาแบบ multidrug resistance (MDR) ผ่าน gene cassettes ใน integron ทำให้เชื้อดื้อยาด้านจุลชีพสูงชันและเป็น การดื้อยาลายชนิดร่วมกันทั้ง broad spectrum beta-lactam, third generation cephalosporins, aminoglycosides, fluoroquinolones และ sulfamethoxazole-trimethoprim ทำให้มีความยากลำบากในการเลือกใช้ยาในการรักษา จึงเกิดแนวคิดที่จะนำสมุนไพรมานำใช้ร่วมกับการใช้ยาต้านจุลชีพเพื่อหวังผลเสริมฤทธิ์กันและลดอุปสรรคการดื้อยา การวิจัยครั้งนี้จึงได้นำสารสกัดแอลกอฮอล์จากผลสมอติ่ง ampicillin และ norfloxacin มาศึกษาฤทธิ์ต้านเชื้อ *E.coli* จำนวน 30 สายพันธุ์ (29 สายพันธุ์สร้าง ESBLs และอีก 1 สายพันธุ์ไม่สร้าง ESBL) พบว่ามีค่า MIC₅₀ เท่ากับ 10 มก/มล, >256 มก/มล และ 64 มก/มล ตามลำดับ เชื้อ *E.coli* ทั้ง 30 สายพันธุ์ตรวจพบ beta-lactamase activity (จากการทดสอบด้วย Nitrocefin base test) และดื้อต่อ ampicillin ในระดับสูง (MIC อยู่ในช่วง 256- >256 มก/มล) มีเพียง 18 สายพันธุ์ (60%) ที่ดื้อทั้ง ampicillin และ norfloxacin และเป็นสายพันธุ์ที่สร้าง ESBLs ทั้งหมด เมื่อประเมินฤทธิ์ร่วมโดยวิธี Checkerboard ใน *E.coli* ทั้ง 30 สายพันธุ์พบว่า การให้สารสกัดแอลกอฮอล์จากผลสมอติ่งร่วมกับ ampicillin ไม่แสดงผลต่อกัน (indifference) แต่การให้สารสกัดแอลกอฮอล์จากผลสมอติ่งร่วมกับ norfloxacin ใน *E.coli* ที่สร้าง ESBLs จำนวน 18 สายพันธุ์ที่ดื้อต่อยาต้านจุลชีพทั้งสองปรากฏว่าให้ผลเสริมฤทธิ์กัน (synergy) 11 สายพันธุ์ (61.11%) และเสริมฤทธิ์บางส่วน (partial synergy) 6 สายพันธุ์ (33.33%) และอีก 1 สายพันธุ์ (5.55%) ให้ผลเพิ่มฤทธิ์ (additive) โดยที่ไม่มีแม้แต่สายพันธุ์เดียวที่ไม่แสดงผลต่อกัน เมื่อนำ *E.coli* ที่สร้าง ESBLs ทั้ง 18 สายพันธุ์ มาศึกษาต่อเพื่อประเมินผลฆ่าเชื้อโดยวิธี Time kill assay พบว่าการใช้สารสกัดแอลกอฮอล์จากผลสมอติ่งเดี่ยว (½ MIC และ 1 MIC) ไม่สามารถฆ่าเชื้อได้ถึง 99% ในขณะที่ใช้ norfloxacin (½ MIC) เพียงอย่างเดียวจะฆ่าเชื้อได้ 99.9% จำนวน 2 สายพันธุ์ตั้งแต่ชั่วโมงที่ 8 แต่อย่างไรก็ตามการใช้สารสกัดแอลกอฮอล์จากผลสมอติ่ง หรือ norfloxacin เดี่ยวๆ เชื้อจะกลับเจริญขึ้นได้อีก (regrowth) ที่เวลา 24 ชั่วโมง เป็นจำนวน 14, 16 และ 12 สายพันธุ์ ตามลำดับ การใช้สารสกัดแอลกอฮอล์จากผลสมอติ่งร่วมกับ norfloxacin ถ้าเพิ่มจำนวนสารสกัดจาก ½ MIC เป็น 1 MIC จะฆ่าเชื้อได้เร็วขึ้นและจำนวนเชื้อที่ถูกฆ่าจะเพิ่มขึ้น เช่น *E.coli* สายพันธุ์ U16 ซึ่งถูกฆ่าได้ 99.9% ที่ 4 ชั่วโมง จะเร็วขึ้นเป็น 2 ชั่วโมงแรก และที่เวลา 24 ชั่วโมง จะมีเชื้อที่ถูกฆ่า 90-99.9% จำนวน 4 สายพันธุ์ และจำนวนเชื้อที่ถูกฆ่า (BA₅₀) ทั้งหมด เท่ากับ (164.75±30.69 logCFU/ml-h) ซึ่งจะเพิ่มจำนวนเป็น 16 สายพันธุ์ และจำนวนเชื้อที่ถูกฆ่าทั้งหมดเพิ่มขึ้นเป็น (175.78±30.14 log CFU/ml-h) เมื่อได้รับสารสกัดแอลกอฮอล์จากผลสมอติ่งเพิ่มขึ้นจาก ½ MIC เป็น 1 MIC ร่วมกับ norfloxacin ½ MIC นอกจากนี้การใช้สารสกัดแอลกอฮอล์จากผลสมอติ่งร่วมกับ norfloxacin จะไม่ทำให้เชื้อ *E.coli* ที่สร้าง ESBLs ทั้ง 18 สายพันธุ์กลับเจริญขึ้นมาอีก ที่ 24 ชั่วโมง จากผลการทดลองแสดงให้เห็นว่า การให้สารสกัดแอลกอฮอล์จากผลสมอติ่งร่วมกับ norfloxacin แสดงฤทธิ์ฆ่าเชื้อได้ดีกว่าการให้ norfloxacin หรือสมุนไพรวัดเดียว เมื่อเปรียบเทียบในด้านจำนวนเชื้อที่ถูกฆ่า ความเร็วในการฆ่าเชื้อ และการที่เชื้อไม่สามารถกลับเจริญขึ้นมาอีก ซึ่งจะช่วยลดอุปสรรคการดื้อยาดังนั้นจึงอาจเป็นอีกทางเลือกหนึ่งในการพัฒนาสารสกัดแอลกอฮอล์จากผลสมอติ่งเป็นยาที่ใช้ร่วมกับ norfloxacin เพื่อรักษาโรคติดเชื้อ *E.coli* สายพันธุ์ที่สร้างเอนไซม์ ESBLs ซึ่งดื้อต่อ ampicillin และ norfloxacin แล้ว

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Extended spectrum beta-lactamase producing *Escherichia coli* is a problem in hospitalized patients worldwide and are increasingly associated with community acquired infection. The most frequent coresistances found in ESBL-producing organisms are broad spectrum beta-lactam, third generation cephalosporins, aminoglycosides and fluoroquinolones. Combination therapy is the other choice in the treatment of ESBL-producing *E.coli* infections with the aim of decreasing the emergence of resistance strains and increasing bacterial killing. Therefore, the purpose of the present study is to determine the susceptibility of alcoholic extract of *T. citrina* ROXB, ampicillin and norfloxacin against 30 *E.coli* (29 ESBL-producing, 1 non- ESBL-producing) strains. The MIC₉₀ values were 10 mg/ml, >256 µg/ml and 64 µg/ml, respectively. All of 30 strains were beta-lactamase positive by Nitrocefin base test. The high ampicillin resistance were detected (MIC range 256- >256 µg/ml). There were 18 strains of ESBL –producing *E.coli* (60%), which were resistant to both ampicillin and norfloxacin. Checkerboard method served to determine the activity of extract in combination with ampicillin and norfloxacin. Combination of the extract with ampicillin showed indifference against 30 *E.coli* strains while norfloxacin plus the extract against 18 strains of ESBL –producing *E.coli* (resistance to both ampicillin and norfloxacin) showed synergistic effect in 11 strains (61.11%), partial synergistic effect in 6 strains (33.33%) and additive effect in 1 strain (5.55%). In the Time kill study using 18 ESBL-producing *E.coli* strains, extract alone (½ MIC, 1 MIC) showed bacteriostatic activity (90% killing), whereas norfloxacin alone (½ MIC) showed bactericidal activity (99.9% killing) in 2 strains at 8 hour of growth. However, the regrowth were observed in 14, 16 and 12 strains at 24 hour by the extract alone or norfloxacin alone. When the concentration of the extract was increased from ½ MIC to 1 MIC and combined with norfloxacin, the bactericidal rate was faster and the number of strains killed was increased. For example, in strain no. U16 had the bactericidal activity was seen at 2 hour instead of 4 hour when using the combination of the extract 1 MIC plus norfloxacin. At 24 hour of growth, the bacteriostatic and bactericidal activity (90-99.9% killing) were observed in only 4 strains [BA₂₄=164.75 ±30.69 log CFU/ml·h] (1/2 MIC of the extract plus norfloxacin), while combined 1 MIC of the extract plus norfloxacin showed the bacteriostatic and bactericidal activity (90-99.9% killing) against 16 strains [BA₂₄=175.78±30.41 log CFU/ml·h]. The results obtained suggested that antibacterial activity of the combination extract plus norfloxacin were higher than the antibacterial activity of each drug. It is concluded that the combination of extract plus norfloxacin could be promising alternatives in the treatment of infections due to ESBL-producing *E.coli* that were resistant to ampicillin and norfloxacin.

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LIST OF ABBREVIATIONS

°C	= degree Celsius
AUBKC	= Area under the bacterial killing regrowth curves
AUC	= Area under the curve
BA24	= Bacteriolytic area of 24 hours
CFU	= Colony forming unit
<i>E.coli</i>	= <i>Escherichia coli</i>
ESBL	= extended-spectrum beta-lactamase
et al.	= et alii (and other peoples)
g	= gram
hr	= hour
L	= Liter
log	= decimal logarithm
MBC	= Minimum bactericidal concentration
MHA	= Mueller-Hinton agar
MHB	= Mueller-Hinton broth
MIC	= Minimum inhibitory concentration
min	= minute
ml	= milliliter
mm	= millimeter
mol	= mole
NCCLS	= The National Committee for Clinical Laboratory Standards
NSS	= Normal saline solution
TSA	= Tryptic Soy Agar
µg	= microgram
µl	= microliter