Antibacterial Activity of Thai Medicinal Plants Pikutbenjakul

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Background: *Bacterial infections caused by resistant strains have been increased dramatically. Pikutbenjakul, a Thai medicinal plant formula containing Piper longum, Piper sarmentosum, Piper interruptum, Plumbago indica and Zingiber officinale have been widely used in Thai traditional medicine.*
Objective: *To determine antimicrobial activity of Pikutbenjakul formula and its components in order to develop the medicinal plants for alternative treatment of bacteria causing diarrhea.*
Material and Method: *Activity of Pikutbenjakul formula and its components was tested using disc diffusion and broth dilution methods against bacteria associated a set of bacteria associated with diarrheal disease including Vibrio cholerae, Vibrio vulnificus, Salmonella, Shigella, Escherichia coli (EIEC, ETEC, EPEC, EAEC and EHEC) and Staphylococcus aureus. The extraction was performed by maceration in 95% ethanol.*
Results: *The results showed all tested strains were susceptible to P. indica while other components were able to inhibit some strains. P. sarmentosum showed antimicrobial activity against Vibrios with the MIC values between 0.625 to ≥ 5mg/ml. P. sarmentosum, P. indica and Pikutbenjakul formulas inhibited the growth of all Vibrios. P. interruptum inhibited V. cholerae serogroups O1 and non-O1/non-O139. P. longum was able to inhibit only two isolates of V. cholerae serogroup O139 (MIC = 1.25 mg/ml) and V. vulnificus (MIC ≥ 5 mg/ml). The activity of Pikutbenjakul containing Zingiber spp. and Pikutbenjakul containing Z. officinal against Vibrios, Shigella spp. and S. aureus was not significantly different. P. indica could inhibit Salmonella (MIC ≥ 5 mg/ml), E. coli (MIC ≥ 5 mg/ml) and S. aureus (MIC = 1.25 mg/ml).*
Conclusion: *The results support the Thai medicinal plants for treatment of diarrhea caused by these bacteria. This study also provides an insightful knowledge on antimicrobial activity which would lead to further development of an effective formula of Pikutbenjakul for diarrheal disease and other infectious diseases in future.*

Keywords*: Antibacterial activity, Pikutbenjakul, Diarrheal disease*

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Pikutbenjakul is a Thai medicinal plant formula containing *Piper longum*, *Piper sarmentosum*, *Piper interruptum*, *Plumbago indica* and *Zingiber officinale*. The components were previously shown their antimicrobial activity such as the extract of *P. indica* had antibacterial activity against *Samonella typhosa* and *Staphylococcus aureus*[1] and *P. longum* exhibited antibacterial activity against *Salmonella typhimurium* and *S. aureus*[2]. Moreover, the fruit part of *P. longum* was previously reported to be able to inhibit *Entamoeba histolytica* causing acute diarrheal disease[3]. However, the antimicrobial activity of Pikutbenjakul formula and its components against bacteria associated with diarrheal disease have not been fully investigated. The bacteria include *Vibrio*s, *Salmonella* spp., *Shigella* spp., *Escherichia coli* including Enterohemorrhagic *E. coli* (EHEC),

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Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC) and *S. aureus*. These bacteria have become resistant to many antibiotics and rapidly spread. The emerging multiresistant strains could be potentially prevented and controlled by monitoring antibiotic resistance and antibiotic usage. Hence, this study aims to investigate the antimicrobial activity of the extracts of Pikutbenjakul formula and its components against the bacteria in order to develop an effective formula for an alternative traditional plant-based medicine treatment of bacterial infections. As a consequence, it would prevent and slow the emergence of resistance among the bacteria.

Material and Method

Thirty-two clinical isolates were collected from Songklanakarind hospital, Thammasat Hospital and Enteric Diseases Department, USAMC-AFRIMS, Thailand. The bacterial strains were *Vibrios* (n = 10): *V. cholera*, *V. vulnificus*; *Escherichia coli* (n = 5): Enterohemorrhagic *E. coli* (EHEC), Enterotoxigenic *Escherichia coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC); *Salmonella* (n = 16): *S. typhi*, *S. typhimurium*; *Shigella* (n = 4): *S. dysenteriae*, *S. flexneri*, *S. boydii*, *S. sonnei* and *Staphylococcus aureus* (n = 1). The components of Pikutbenjakul used in this study were fruit of *P. longum*, root of *P. sarmentosum* and *P. indica*, stem of *P. interruptum*, rhizome of *Z. officinale* and *Zingiber* spp. Either *Zingiber officinale* or *Zingiber spp.* were added in Pikutbenjakul formulas designated PBK1 and PBK2, respectively. The extraction of Pikutbenjakul was performed by maceration in 95% ethanol. The extracts were then dissolved in 1% DMSO for antimicrobial assay. The antimicrobial activities were determined by disc diffusion method according to NCCLS (2004)[4] for screening and using microtitre plate-based antibacterial assay described previously[5] for determination of minimal inhibitory concentration (MIC) of the extracts against the bacteria. The concentration of Pikutbenjakul formulas and its components was 5 mg/ml per disc. The MIC test was modified by adding resazurin after incubating at 35-37°C for 16-18 hrs and incubated further for 2 hrs. The inoculum was prepared equivalent to a 0.5 McFarland standard by densitometer (GrantBio, England). Ampicllin and DMSO were used as positive and negative control, respectively. Viability bacterial control was also included. The antimicrobial tests were performed in triplicate.

Results

The extracts from maceration in 95% alcohol were shown to be more effective than the extracts from water extraction process except *P. interruptum* and *P. indica*. The two components demonstrated the antimicrobial activities against *V. vulnificus* and *V. cholerae* non-O1/non-O139, respectively (data not shown). In addition, *P. indica* showed the large inhibition zone when tested with *S. aureus* and some *Vibrios* (Table 1). The activity of Pikutbenjakul formula containing *Zingiber spp.* (PBK1) and Pikutbenjakul formula containing *Z. officinale* (PBK2) against *Vibrio spp., Shigella spp.* and *S. aureus* is not significantly different. Both formulas showed no activity against *E. coli* tested in this study (Table 1).

The MIC values showed that all tested strains were susceptible to *P. indica* while other components were able to inhibit only some strains. For example, *P. sarmentosum* exhibited antimicrobial activity against only *Vibrios* with the MIC values between 0.625 to ≥ 5 mg/ml. *P. samentosum*, *P. indica* and Pikutbenjakul formulas inhibited the growth of all the isolates of *Vibrio*s. *P. interruptum* showed antimicrobial activity against *V. cholerae* serogoup O1 and non-O1/non-O139. *P. longum* was susceptible to only two isolates of *V. cholerae* serogoup O139 (MIC = 1.25 mg/ml) and *V. vulnificus* (MIC ≥ 5 mg/ml). The MIC values of *P. indica* against *Salmonella* spp. and *E. coli* including EIEC, ETEC, EPEC, EAEC and EHEC were ≥ 5 mg/ml while *S. aureus* demonstrated the MIC value of 1.25 mg/ml (Table 3).

Discussion

P. indica was shown to be the most effective component of Pikutbenjakul inhibiting all tested strains including *Vibrio* spp., *Salmonella* spp., *Shigella* spp., *E. coli* (EIEC, ETEC, EPEC, EAEC and EHEC) and *S. aureus*. The obtained results supported antimicrobial activity of the extracts in previous reports. For example, the extract from *P. indica* using maceration in 95% ethanol was able to inhibit *Samonella typhosa*[1]. However, the MIC values of *P. indica* against *Salmonella*, *Shigella* and *E. coli* in this study were relatively high. In addition, both formulas of Pikutbenjakul were either no activity or high values of MIC against the bacteria and also showed higher MIC value than single crude extracts of each component as mentioned. It is suggested to adjust the proportions of Pikutbenjakul components in order to obtain the most efficient antimicrobial activity and to avoid antagonistic effects among the extracts which may occur.

Table 1. Antimicrobial activity of extracts from components and Pikutbenjakul formulas by disk diffusion method

Extract		Inhibition zone (mm)				
		Salmonella	*Vibios*	*E. coli*	*S. aureus*	*Shigella*
Piper interruptum						
	EtOH	0	7-12.7	0	0	0
	H_2O	0	7.7	0	0	0
Piper longum						
	EtOH	0	7-11.3	0	0	0
	H_2O	0	0	0	0	0
Piper sarmentosum						
	EtOH	0	7.3-13.7	0	0	8
	H_2O	0	0	0	0	0
Plumbago indica						
	EtOH	8-9.3	16.7-28.3	7.7-10.3	28.7	8.7-17.3
	H_2O	0	14-15	0	0	7.7-8
Zingiber spp.						
	EtOH	0	8-11.3	0	0	0
	H_2O	0	0	0	0	0
Zingiber officinale						
	EtOH	0	7-12.7	0	0	7.7
	H_2O	0	0	0	0	0
Pikutbenjakul containing *Zingiber spp.*						
	EtOH	0	7.3-14.3	0	14.7	9-11
	H_2O	0	0	0	0	0
Pikutbenjakul containing *Zingiber officinale*						
	EtOH	0	7.3-18	0	14	9-11
	H_2O	0	0	0	0	0

Sawangjaroen (2004) showed that *P. longum* fruit had better effect on killing *Entamoeba histolytica* associated with chronic diarrhea in mice compared to *P. sarmentosum*[3]. The antimicrobial activity of piperine, a pure compound from root of *P. longum* against *S. aureus* was reported previously[2]. Moreover, *Pseudomonas aeruginosa, Bacillus cereus, Serratia marcescens, E. coli, Shigella dysenteriae, Salmonella typhi, S. aureus* and *Klebsiella pneumoniae* were also susceptible to piperine extracted from root of *P. longum*[6]. The previous studies as mentioned are supporting *P. longum* as a potentially effective candidate for Pikutbenjakul formula. However, the extracts of *P. longum* has limited activity against some particular isolates. The use of different parts of the component for better inhibition against the causative agents associated with diarrheal disease should be further investigated. In addition, antimicrobial activity of pure extract is suggested for future studies in order to obtain more insightful detail knowledge for developing an effective Pikutbenjakul formula.

The isolates from clinical specimen in this study were found to be resistant to many antibiotics such as *E. coli* strains were resistant to azithromycin, ampicillin, chloramphenicol, gentamicin, sulfamethozazole with trimethoprim and tetracycline. Moreover, *Salmonella* and *Shigella* were resistant to ampicillin, chloramphenicol, gentamicin, sulfamethozazole with trimethoprim and tetracycline (data not shown). Hence, the medicinal plants would be considered as an alternative treatment for bacterial infections associated with diarrheal disease.

Conclusion

The obtained results support the Thai medicinal plants for treatment of diarrheal disease caused by the bacteria. This study provides an insightful knowledge on antimicrobial activities of each

Table 3. Antimicrobial activity of Pikutbenjakul extracts by broth dilution method

Extract	MIC (mg/ml)				
	Salmonella	*Vibios*	*E. coli*	*S. aureus*	*Shigella*
Piper interruptum					
EtOH	-	0.625-5	-	-	-
H_2O	-	5	-	-	-
Piper longum					
EtOH	-	1.25-> 5	-	-	-
H_2O	-	-	-	-	-
Piper sarmentosum					
EtOH	-	0.625-> 5	-	-	> 5
H_2O	-	-	-	-	-
Plumbago indica					
EtOH	5-> 5	0.156-5	5->5	1.25	2.5-5
H_2O	-	> 5	-	-	> 5
Zingiber spp.					
EtOH	-	5-> 5	-	-	-
H_2O	-	-	-	-	-
Zingiber officinale					
EtOH	-	< 0.039-> 5	-	-	> 5
H_2O	-	-	-	-	-
Pikutbenjakul containing *Zingiber spp.*					
EtOH	-	2.5-> 5	-	2.5	> 5
H_2O	-	-	-	-	-
Pikutbenjakul containing *Zingiber officinale*					
EtOH	-	2.5-> 5	-	5	> 5

extracts of Pikutbenjakul and formulas leading to further develop an effective formula of Pikutbenjakul for other infectious diseases in future.

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ฤทธิ์ต้านเชื้อแบคทีเรียของสมุนไพรไทยตำรับพิกัดเบญจกูล

สุมาลี คอนโด, ชิษณุชา สัตพนพันธุ์, เสาวลักษณ์ พงษ์ไพจิตร, อภิชัย ศรีจันทร์, อรุณพร อิฐรัตน์

ภูมิหลัง: *การติดเชื้อแบคทีเรียที่เกิดจากเชื้อดื้อยาพบว่ามีการเพิ่มจำนวนมากขึ้นอย่างรวดเร็ว ตำรับพิกัดเบญจกูลมีส่วนประกอบของพืชสมุนไพรไทย คือผลดีปลี, รากช้าพลู, เถาสะค้าน, รากเจตมูลเพลิงแดง และเหง้าขิงแห้ง ซึ่งใช้กันอย่างแพร่หลายในการแพทย์แผนไทย*

วัตถุประสงค์: *ศึกษาฤทธิ์ของสมุนไพรทั้งตำรับ และส่วนประกอบของสมุนไพรแต่ละชนิดเพื่อพัฒนาสมุนไพรไทยให้เป็นทางเลือกสำหรับการรักษาโรคอุจจาระร่วงที่เกิดจากการติดเชื้อ*

วัสดุและวิธีการ: *ศึกษาฤทธิ์ของสมุนไพรทั้งตำรับ และส่วนประกอบของสมุนไพรแต่ละชนิดด้วยวิธี disc diffusion และ broth dilution ต่อเชื้อแบคทีเรียที่ก่อโรคอุจจาระร่วงได้แก่ Vibrio cholerae, Vibrio vulnificus, Salmonella, Shigella, E. coli (EIEC, ETEC, EPEC, EAEC และ EHEC) และ S. aureus การสกัดสมุนไพรทำโดยใช้วิธีสกัดด้วย 95% เอธานอล*

ผลการศึกษา: *พบว่าเชื้อที่ทดสอบทั้งหมดมีความไวต่อสารสกัดเจตมูลเพลิงแดง ในขณะที่สารสกัดจากส่วนอื่นสามารถยับยั้งเชื้อได้บางชนิด ช้าพลูแสดงฤทธิ์การยับยั้งเชื้อกลุ่ม Vibrios โดยมีค่า MIC ระหว่าง 0.625 to ≥ 5 mg/ml และพบว่าทั้งสารสกัดช้าพลู เจตมูลเพลิงแดง และตำรับพิกัดเบญจกูล สามารถยับยั้งเชื้อกลุ่ม Vibrios ได้สารสกัดสะค้านยับยั้งเชื้อ V. cholerae serogroups O1 และ non-O1/non-O139 สารสกัดดีปลีสามารถยับยั้งเชื้อได้เฉพาะ V. cholerae serogroups O139 (MIC = 1.25 mg/ml) และ V. vulnificus (MIC ≥ 5 mg/ml) ฤทธิ์ต้านเชื้อ Vibrio spp., Shigella spp. และ S. aureus ของตำรับพิกัดเบญจกูลที่มี ส่วนประกอบของขิงแห้งไม่แตกต่างจากสารสกัดตำรับพิกัดเบญจกูลที่มีขิง สารสกัดเจตมูลเพลิงแดงสามารถ ยับยั้งเชื้อเหล่านี้ได้โดย Salmonella และ E. coli มีค่า MIC ≥ 5 mg/ml ขณะที่ S. aureus มีค่า MIC เพียง 1.25 mg/ml*