Antimicrobial Activity of Rice Bran Extracts for Diarrheal Disease

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Background: *Rice bran showed antioxidative, antimutagenic, carcinogenic and antibacterial activities in previous reports. The rice bran has been recently used as a natural source of health food for several diseases such as diabetes, atherosclerosis and cancer. Severe diarrheal disease due to food-borne contamination of bacteria resulted from the bacteria have become resistant to many antibiotics. Hence, early treatment of diarrhea using natural food containing antibacterial activity to prevent progression of severe symptoms will be beneficial.*
Objective: *To investigate antimicrobial activity of rice bran extracts against bacteria causing diarrheal disease.*
Material and Method: *Bacterial strains isolated from patients include Vibrio cholerae, Vibrio vulnificus, Salmonella spp, Shigella spp, Escherichia coli (ETEC, EHEC, EAEC, EPEC, EIEC) and Stahylococcus aureus. Rice bran was extracted by five different extraction techniques. The antimicrobial activity was performed by disk diffusion and broth dilution methods.*
Results: *The results showed that rice bran extracts using different techniques of extraction were able to inhibit the growth of test strains. Rice bran extracts exhibited the most effective antibacterial activity against V. cholerae O139 with MIC value of 0.976 mg/ml. Using ethanol and supercritical techniques, Sang-Yod rice bran showed better antibacterial activity than Jasmine rice bran. In the present study, the MIC values of rice bran extracts against all tested strains except V. cholerae O139 and S. aureus were between 7.812 to 31.25 mg/ml.*
Conclusion: *The results of present study provide insighful basic knowledge which would lead to develop rice bran extracts for effective treatment of diarrheal disease causing by bacteria including resistant strains. The rice bran extracts used against bacterial infection will be an alternative remedy in order to reduce the incidence of antibiotic resistance in future.*

Keywords: *Diarrheal disease, Vibrio cholerae, Vibrio vulnificus, Salmonella spp, Shigella spp, E. coli (ETEC, EHEC, EAEC, EPEC, EIEC) and Stahylococcus aureus, Rice bran extracts*

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Rice bran contains several nutritious ingredients and is a rich source of bioactive phytochemicals. It provided health beneficial effects and has been used as a natural source of healthy food for several diseases such as diabetes[1], atherosclerosis[2] and cancer[3]. Extracts from Rice bran showed various activities such as antioxidative[4,5], antimutagenic, carcinogenic and antibacterial[3,6]. Kawakami (2006) reported that washed Japanese rice was able to inhibit *Helicobacter pyroli* that cause gastroduodenal disease[7]. Moreover, heat-stabilized defatted rice bran (HDRB) extract showed growth inhibition of *Listeria monocytogenes*, *Escherichai coli* O157: H7 and *Salmonella* Thyphimurium[8].

Acute diarrhea is cured by properly compensation of water and mineral to prevent loosing water and mortality. The oral rice water (RW) was proposed to be an antidiarrheal agent as its convenience and effectiveness. The RW showed the efficacy control of diarrhea equivalent to the standard World Health Organization oralyte solution (WOS). However, the RW revealed more rapid compensation of loosing water[9].

Moreover oral rice-based rehydration solution (SRO) was an alternative treatment of malnutrition with

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acute diarrhea in children and shortened the duration of diarrhea symptom[10]. Other similar study indicated that treatment of infant with acute diarrhea using rice-based oral rehydration solution (R-ORS) lowered quantity of stool (35%) comparing to control group with glucose-based oral rehydration solution (G-ORS)+ soy-based, lactose-based formula (SF). In addition consuming porridge or R-ORS immediately after loosing water showed the same outcome as treatment with R-ORS alone for 24 hrs followed by SF. The safety and efficacy of ready-to-use, premixed, R-ORS had been studied for reduction of intravenous fluid therapy[11].

Many causative agents produced acute diarrhea include bacteria, parasite, virus and others. The bacteria including *E. coli*, *Vibrio cholerae*, *Shigella* spp, *Salmonella* spp, *E. coli* O157:H7, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens, V. parahaemolyticus* usually are food-borne contamination. These strains have become resistant to many antibiotics. It is markedly important to control the spread of an emerging resistant strain that may take place more in future. To develop rice bran extracts for an alternative antibacterial treatment, antibacterial activity is therefore tested in the present study in a hope to overcome new emerging resistant strains. In addition further development of rice bran products for treatment diarrheal disease caused.

Material and Method

Bacterial strains were clinically isolated from Songklanakarind Hospital, Thammasat hospital and Enteric Diseases Department, USAMC-AFRIMS, Thailand. The bacterial strains were *Vibrio cholera*, *V. vulnificus*; *E. coli* including Enterohemorrhagic *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC), *Salmonella* Typhi, *S.* Typhimurium, *Shigella dysenteriae, S. flexneri*, *S. boydii*, *S. sonnei* and *Staphylococcus aureus*. The rice bran of Jasmine rice, milled but unpolished rice (Klong Rice) and Sung-Yod rice tested in the present study were listed in Table 1. The extraction was performed by maceration in 95% ethanol; supercritical extraction; cold press process; decoction followed by freeze dry and washing with water followed by freeze dry. Antimicrobial activities were determined by disk diffusion method[12] and modified microtitre plate-based antibacterial assay as previous report[13]. Minimum Bactericidal Concentration (MBC) was also performed. The inoculum was prepared equivalent to a 0.5 McFarland standard. 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 MIC and MBC of rice bran extracts were at the same concentration (data not shown). The results of antibacterial activity of rice bran extracts against bacteria causing diarrheal disease were shown in Table 2. MIC values of rice bran extracts using different techniques showed the best antibacterial activity against *V. cholerae* and *S. aureus* with MIC value of $\leq$ 0.976 mg/ml. The MIC of rice bran extracts ranged from $\leq$ 0.976 mg/ml to 62.5 mg/ml. Extraction by maceration with 95% ethanol and supercritical techniques of Sung-Yod rice bran showed better activity than Jasmine rice bran for growth inhibition of *S. aureus*. The antibacterial activity of rice bran using various techniques in the present study against *Salmonella*

Table 1. Code of rice bran extracts using different techniques of extraction

Code	Extracts
1. SYBMEt	Maceration in 95% ethanol of Sung-Yod rice bran
2. MLGMEt	Maceration in 95% ethanol of milled but unpolished Jasmine rice bran
3. MLBMEt	Maceration in 95% ethanol of Jasmine rice bran
4. MLWMEt	Maceration in 95% ethanol of Jasmine rice
5. SYBSUP	Supercritical extraction of Sung-Yod rice bran
6. MLBSUP	Supercritical extraction of Jasmine rice bran
7. MLBEX	Cold press process Jasmine rice bran oil
8. SYBEX	Cold press process Sung-Yod rice bran oil
9. SYBBOIL	Decoction and freeze dried Sung-Yod rice bran
10. MLBBOIL	Decoction and freeze dried Jasmine rice bran
11. MLWW	Washed Jasmine rice bran with water and freeze dried
12. MLGW	Washed milled but unpolished Jasmine rice with water and freeze dried

Table 2. Minimum Inhibitory concentration (MIC) and Minimum Bactericidal concentration (MBC) of rice bran extracts against pathogenic bacteria causing diarrheal disease

Rice bran extract	MIC/MBC (ms/ml)					
	Salmonella spp.	*Shigella* spp.	*E. coli*	*V. cholerae*	*V. vulnificus*	*S. aureus*
SYBMEt	15.62	15.62	15.62	< 0.976-15.62	7.812	1.95
MLGMEt	15.62	15.62	15.62	< 0.976-31.25	15.62	31.25
MLWMEt	15.62	15.62	15.62	< 0.976-31.25	15.62	31.25
MLBMEt	15.62	15.62	15.62	< 0.976-31.25	7.812	31.25
SYBSUP	15.62	15.62	15.62	< 0.976-31.25	7.812	< 0.976
MLBSUP	15.62	15.62	15.62	< 0.976-31.25	15.62	3.9
MLBEX	15.62	15.62	15.62	< 0.976-31.25	15.62	15.62
SYBEX	15.62	15.62	15.62	< 0.976-31.25	15.62	< 0.976
SYBBOIL	15.62	15.62	15.62	< 0.976-31.25	15.62	< 0.976
MLBBOIL	15.62	15.62	15.62	< 0.976-31.25	15.62	62.5
MLGW	15.62	15.62	15.62	15.62-31.25	15.62	62.5
MLWW	15.62	15.62	15.62	15.62-31.25	15.62	62.5

spp, *Shigella* spp, *E. coli*, *V. vulnificus* and *V. cholerae* except *V. cholerae* O139 showed that the MIC values ranged from 7.812 mg/ml to 31.25 mg/ml.

Comparison of antibacterial activity of Sung-Yod rice bran (SYBBOIL) and Jasmine rice extracts (MLWW**)** by decoction showed growth inhibition of only *S. aureus* and *V. cholerae* O139. The SYBBOIL had better activity than the MLWW (Table 2). The activity of SYBBOIL was not significant different in growth inhibition of other bacteria. Washed Jasmine rice bran with water followed by freeze dry (MLWW) and washed milled but unpolished Jasmine rice bran with water followed by freeze dried (MLGW) were no significant differences of antibacterial activity against the bacteria tested in the present study.

Analysis of the obtained results among different serogroup and species of *Vibrios* revealed that rice bran extracts established more efficient antibacterial activity against *V. cholerae* O139 than other serogroups (Fig. 1). The MIC of the extracts against *V. vulnificus* (MIC = 7.812-31.25 mg/ml) showed no significant difference with all *V. cholerae* strains except *V. cholerae* O139 (MIC $\leq$ 0.976 mg/ml).

Discussion

The antibacterial activity of rice bran extracts were observed in the present study in addition to other properties such as antioxidative, antimutagenic and carcinogenic activities as reported previously[3]. The obtained results showed that the rice bran extracts established the property of inhibition and killing of the pathogenic bacteria that pose serious problem in human particularly diarrheal disease.

The effective microorganism fermentation extract (EM-X), a refreshment beverage was modified so called EM-YU contained local rice bran, seaweed, and kiwifruit with effective microorganisms showed several activities including antioxidant, antibacterial, tyrosinase inhibition activities and biofilm inhibition activity of pathogenic bacteria[6]. As the results of the present study observed for antibacterial activity against pathogenic bacteria that caused diarrheal disease, it is indicated that our local rice bran extracts has the potentially beneficial property as a rich source.

Rice-fluid derived from Japanese raw rice demonstrated strong bactericidal activities only against *Helicobacter pylori*, *Streptococcus pneumoniae* and *Campyrobacter jejuni* strains. It was suggested that it possibly contained the compound containing bactericidal agents. Moreover, the report indicated that the rice-fluid had no bactericidal activity against normal

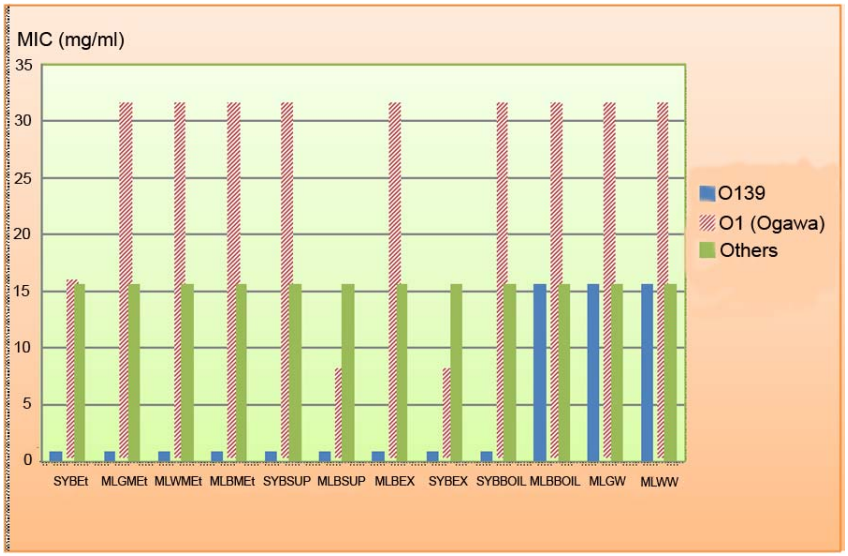

Fig. 1 Minimum inhibitory concentration (MIC) of rice bran extracts against different serogroups of *V. cholerae*

flora found in gastrointestinal tract. The rice fluid was therefore potential used as a new therapeutic agent against *H. pylori* infection[7]. The present study demonstrated that the rice bran extracts had antibacterial activity against pathogenic bacteria that cause acute diarrhea. It is indicated that the different rice bran extracts were efficient in killing and inhibiting of various pathogenic bacteria and resistant strains. As the obtained results, the rice bran extracts are considered to be a new source for treatment of diarrheal disease. This observation provides a promising data for further approach of developing alternative drug of choices using natural resource like rice bran for other bacterial infections particularly due to resistant bacterial strains. However, active compounds of the effective extracts are necessary to be investigated for specific marker of the antibacterial activity against the pathogenic bacteria. The development of rice bran extracts for alternative medicine will lead to the reduction and control of an emerging resistant strain due to an over use of antibiotics.

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Potential conflicts of interest

None.

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ฤทธิ์ต้านแบคทีเรียก่อโรคท้องร่วงของสารสกัดรำข้าว

สุมาลี คอนโด, รัตนา เตียงทิพย์, ดรุณี ศรีชนะ, อรุณพร อิฐรัตน์

ภูมิหลัง: *จากรายงานวิจัยแสดงคุณสมบัติรำข้าวในการต้านอนุมูลอิสระ ต้านการกลายพันธุ์ ต้านมะเร็ง และต้านแบคทีเรีย รำข้าวเป็นแหล่งอาหารธรรมชาติที่ใช้กับโรคต่างๆ เช่น โรคเบาหวาน โรคหลอดเลือดแข็งตัว โรคมะเร็ง โรคท้องร่วง จากสาเหตุการปนเปื้อนของแบคทีเรียในอาหาร มีการดื้อยาปฏิชีวนะหลายชนิด ดังนั้นการรักษาอาการท้องร่วง ในระยะเริ่มต้นด้วยการใช้อาหารที่มีฤทธิ์ต้านเชื้อจึงเป็นประโยชน์ เพื่อป้องกันการดำเนินอาการของโรคเข้าสู่ระยะที่รุนแรง*

วัตถุประสงค์: *เพื่อศึกษาฤทธิ์ต้านเชื้อแบคทีเรียก่อโรคท้องร่วงของสารสกัดรำข้าวโดยใช้วิธีสกัดที่แตกต่างกัน*

วัสดุและวิธีการ: *เชื้อแบคทีเรียที่ใช้ในการทดสอบครั้งนี้ เป็นเชื้อที่แยกได้จากผู้ป่วยได้แก่ Vibrio cholerae, Vibrio vulnificus, Salmonella spp, Shigella spp, Escherichia coli (ETEC, EHEC, EAEC, EPEC, EIEC) และ Stahylococcus aureus สกัดรำข้าวด้วยวิธีที่แตกต่างกัน 5 วิธี การทดสอบฤทธิ์ต้านเชื้อโดยวิธี disk diffusion และ broth dilution*

ผลการศึกษา: *ผลของสกัดรำข้าวจากการสกัดด้วยวิธีที่แตกต่างกันสามารถยับยั้งการเจริญเติบโตของเชื้อแบคทีเรียที่ทดสอบ รำข้าวสกัดแสดงผลของฤทธิ์ต้านเชื้อที่มีประสิทธิภาพสูงสุดต่อเชื้อ V. cholerae O139 โดยมีค่าความเข้มข้นต่ำสุดที่สามารถยับยั้งเชื้อแบคทีเรียได้เท่ากับ 0.976 มิลลิกรัมต่อมิลลิลิตร รำข้าวสังหยดที่สกัดด้วยแอลกอฮอล์และวิธี supercritical แสดงผลของฤทธิ์ต้านเชื้อได้ดีกว่าที่สกัดจากรำข้าว หอมมะลิ ในการศึกษานี้พบว่า ค่าความเข้มข้นต่ำสุดที่สามารถยับยั้งเชื้อแบคทีเรียที่ทดสอบ ยกเว้น V. cholerae O139 และ S. aureus มีค่าระหว่าง 7.812 มิลลิกรัมต่อมิลลิลิตรและ 31.25 มิลลิกรัมต่อมิลลิลิตร*

สรุป: *จากผลการศึกษานี้จะนำไปสู่การพัฒนาสารสกัดรำข้าว เพื่อรักษาโรคท้องร่วงที่เกิดจากการติดเชื้อแบคทีเรีย รวมถึงเชื้อดื้อยาปฏิชีวนะ การสกัดรำข้าวที่มีฤทธิ์ต้านเชื้อจะเป็นยารักษาทางเลือกเพื่อลดอุบัติการณ์การดื้อยาปฏิชีวนะของแบคทีเรียก่อดรคต่อไปในอนาคต*