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Antidiarrheal Activity of The Combination of Ethanol Extracts of Turmeric Rhizomes, Gall oak Leaves, Guava Leaves and Meniran Herbs

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ABSTRACT

Diarrheal disease is an endemic disease in Indonesia and also a potential disease that can cause death. In practice, the use of traditional medicines derived from plants still being considered by society due to its viability, economic factors and safety reasons. Several plants worked as antidiarrheal agents with the mechanism actions of (1) antibacterial (inhibited the growth of *Escherichia coli*, *Shigella dysenteriae*, *Shigella flexneri, Staphylococcus aureus* and *Salmonella* Typhi); (2) reduced the contraction of intestinal as results in the reduction of pain and the reduction of frequency of defecation or; (3) both mechanism of actions. Thus the purpose of the present study is to evaluate antidiarrheal activity of the combination of plant extracts which have been proved to have antidiarrheal activity. Turmeric (*Curcuma domestica*) rhizomes, Gall oak (*Quercus lusitanica*) leaves, Guava (*Psidium guajava*) leaves and Meniran (*Phyllanthus niruri*) herbs were used for this combination. The purpose of this combination was to minimize the doses (based on literature reviews Turmeric at 5% and Meniran 10% inhibited the growth of *Escherichia coli* and *Staphylococcus aureus*, meanwhile guava leaves at 150 mg/kg BW and Gall oak leaves at 10% reduced diarrhea in animal tested). Maceration was chosen for the extraction method using ethanol 96% as the solvent. Protection and transit intestinal method with loperamide HCl as comparison drug and castor oil for the induction of diarrhea were used for antidiarrheal activity, meanwhile diffusion and dilution methods using, inhibition zones, minimum inhibition concentration and minimum bactericidal concentration parameters were used for antibacterial activity. Tetracycline HCl with the concentration of 30 µg was used for the control. The results revealed that the combination of plant extract with the doses of 200 mg/KgBW (1:1:1:1) reduced diarrhea in mice with relaxation of smooth muscle and showed weak antibacterial effects on *Eschericia coli* and *Staphylococcus aureus* but strongly inhibited the growth of *Salmonella* Typhi.

INTRODUCTION

Diarrhea is an alteration in normal bowel movement and is characterized by an increase in the water content, volume, or frequency of stools (Guerrant et al. 2001). Diarrheal diseases are a major problem in Third World countries and are responsible for the death of millions of people each year (Shoba and Thomas 2001). Diarrheal disease is an endemic disease in Indonesia and also a potential disease that can cause death. In practice, the use of traditional medicines derived from plants still being considered by society due to its viability, economic factors and safety reasons. Several plants worked as antidiarrheal agents with the mechanism actions of (1) antibacterial (inhibited the growth of *Escherichia coli*, *Shigella dysenteriae*, *Shigella flexneri, Staphylococcus aureus* and *Salmonella* Typhi); (2) reduced the contraction of intestinal as results in the reduction of pain and the reduction of frequency of defecation or; (3) both mechanism of actions. The antibacterial activity include the potency of diminished toxin caused by bacteria, especially bacteria that caused food poisioning. Thus the purpose of the present study is to evaluate antidiarrheal activity of the combination of plant extracts which have been proved to have antidiarrheal activity. Turmeric (*Curcuma domestica*) rhizomes, Gall oak (*Quercus lusitanica*) leaves, Guava (*Psidium guajava*) leaves and Meniran (*Phyllanthus niruri*) herbs were used for this combination. The purpose of this combination was to minimize the doses being used. Turmeric at 5% and Meniran 10% inhibited the growth of *Escherichia coli* and *Staphylococcus aureus*, meanwhile guava leaves at 150 mg/kg BW and Gall oak leaves at 10% reduced diarrhea in animal tested (Dzulkarnain et al. 1978; Adyana et al. 2004; Ximenes 2010; Desfita 2011). Protection and transit intestinal method with loperamide HCl as comparison drug and castor oil for the induction of diarrhea were used for antidiarrheal activity, meanwhile diffusion and dilution methods using inhibition zones, minimum inhibition concentration and minimum bactericidal concentration parameters were used for antibacterial activity. Tetracycline HCl with the concentration of 30 µg was used for the control.

METHODS

Drugs and chemicals

Ethanol (CV. Lestari, Surabaya, Indonesia), Loperamide (Kimia Farma, Surabaya, Indonesia) was purchase from local pharmacy, castor oils (Brataco, Surabaya, Indonesia), Nutrient Broth (E.Merck, Germany), Nutrient Agar (E. Merck, Germany)

Plant materials

Turmeric (*C. domestica*) rhizomes, Gall oak (*Q. lusitanica*) leaves, Guava (*P.guajava*) leaves and Meniran (*P niruri*) herbs were obtained in dried powder form from International Herbs Laboratories research.

Animal and Bacteria tested

Mice used for the animal tested were purchased from Animal Laboratories at Faculty of Pharmacy Widya Mandala Catholic University. *S. aureus*, *E. coli* and *Salmonella* Thypi were obtained from Microbiology Laboratories at Faculty of Pharmacy Widya Mandala Catholic University.

Preparation of extracts

Each dried powder of Turmeric (*C. domestica*) rhizomes, Gall oak (*Q. lusitanica*) leaves, Guava (*P. guajava*) leaves and Meniran (*P. niruri*) herbs was soaked in alcohol 96% and leave it for overnight then filtered and evaporated using water bath to dryness. The thick extracts were stored in desiccators until further use.

Antidiarrheal Activity – Protection Method

Animals were divided into three groups of five animals in each group. Group I received normal saline (2ml/KgBW) and served as control. Groups II received standard drug (loperamide 2 mg/kg) and served as standard. Group III received the combination of ethanol extract of Turmeric (*C. domestica*) rhizomes, Gall oak (*Q. lusitanica*) leaves, Guava (*P. guajava*) leaves and Meniran (*P. niruri*) herbs with the doses of 200 mg/KgBW (1:1:1:1). Diarrhea was induced in all the overnight fasted animals by administering 1 ml of castor oil orally. The test extracts and the standard drug were administered one hour prior to the treatment of castor oil. Each mice was housed separately and observed for diarrheal episode, for a period of 4 hours. During that period, number and weight of diarrheal feces were taken and noted at every half an hour. The total number of diarrheal feses and percent protection was calculated. The anti-diarrheal activity was determined in terms of percentage protection. The data of stool weight was expressed as Mean ± SEM (Awounters et al. 1978).

Antidiarrheal Activity – Transit Intestinal Method

The mice were divided into three groups of five animals each and fasted for 18 h but water was freely provided. The first group (control group) received orally normal saline (2 ml/kg BW), Group II receive standard drug (loperamide 2 mg/KgBW) while the third group was given orally the combination of plant extract in doses of 200 mg/kg BW (1:1:1:1). Thirty minutes later, each animal was given 1 ml of charcoal meal (10% activated charcoal in 0.5% gum acacia) via the oral route. All animals were sacrificed 30 min thereafter, and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum was measured and expressed as percentage of distance moved (Mascolo 1994).

Antibacterial Activity – Inhibition zones Determination by Well Diffusion Method

Ten ml of suspension contained $1.5x10^8$ CFU/ml was poured uniformly to solidified 20 mL Nutrient Agar (NA) and the inoculums was allowed to dry for 5 min. Holes of 6 mm in diameter were made in the seeded agar. Aliquot of 20 µl from the combination of plant extract (10.000 ppm, 1:1:1:1) and tetracycline (30 µg) were added into each well on the seeded medium and allowed to stand on the bench for 1 h for proper diffusion and thereafter incubated at 37°C for 24 h. The resulting inhibition zones were measured in millimeters (mm). All the tests were performed in duplicate. Tetracycline (30 µg) served as positive controls.

Antibacterial Activity – MIC Determination by Microdilution Method

The 96-well plates were prepared by dispensing 50 µl of Nutrient Broth, into each well. A 50 µl from the stock solution of tested extracts (concentration of 10.000 ppm) was added into the first row of the plate. Then, twofold, serial dilutions were performed by using a micropipette. The obtained concentration range was from 5000 – 9.77 ppm, and then added 10 µl of inocula to each well except a positive control (inocula were adjusted to contain approximately $1.5x10^5$ CFU/mL. Plant extract with media was used as a positive control and inoculum with media was used as a negative control. The test plates were incubated at 37°C for 18 h. After 18 h 50µl of a 0.01% solution of 2, 3, 5-triphenyl tetrazolium chloride (TTC) was added to the wells and the plate was incubated for another hour. Since the colorless tetrazolium salt is reduced to red colored product by biological active bacteria, the inhibition of growth can be detected when the solution in the well remains clear after incubation with TTC. MIC was defined as the lowest sample concentration showing no color change (clear) and exhibited complete the inhibition of growth (Abu-Shanab et al. 2004)

Antibacterial Activity – MBC Determination by Agar Wells Diffusion Method

The lowest sample concentration showing no growth of bacterial tested from MIC determination used as the concentration sample for this method. Aliquot of 10 µl from the sample were spread on the surface of plate containing Nutrient agar. The plate was incubated at 37°C for 24 h. The presence of colonies was considered a evidence of bacteriostatic action, while the absence of colonies indicated bactericidal activity. All the tests were performed in duplicate.

RESULTS AND DISCUSSION

Our results showed that the combination of extract inhibited castor oil-induced diarrheoa in mice, even though not powerful as loperamid (Table 1). Several mechanisms had been previously proposed to explain the diarrheal effect of castor oil. These include inhibition of intestinal Na^+ K^+ ATPase activity, thus reducing normal fluid absorption (Gaginella and Bass 1978), activation of adenylate cyclase or mucosal cAMP-mediated active secretion (Capasso et al. 1994) stimulation of prostaglandin formation (Galvez et al. 1993) and platelet activating factor (Pinto et al. 1992). Most recently nitric oxide has been claimed to contribute to the diarrheal effect of castor oil (Mascolo *et al*.1996). However, it is well documented that castor oil produces diarrhoea due to its most active component ricinoleic acid through a hypersecretory response (Ammon et al. 1974). Therefore it can be assumed that the antidiarrheal action of the extract was mediated by an antisecretory mechanism. Furthermore, the combination of ethanol extract significantly reduced intestinal transit as evidenced by the decrease in the distance traveled by charcoal meal (Table 2). These results also show that the extract suppressed the propulsion of charcoal meal thereby increasing the absorption of water and electrolytes. Antidiarrheal properties of the combination of plant extract was found to be due to tannins (Gall oak (*Q. lusitanica*) leaves and Guava (*P. guajava*) leaves),

Table 1. Effect of combination of extracts Turmeric (*C.domestica*) rhizomes, Gall oak (*Q. lusitanica*) leaves, Guava (*P. guajava*) leaves and Meniran (*P. niruri*) herbs on castor oil induced diarrhea in mice

Group Treatment (*p.o*)	Total number of diarrheal feces (g)	% Inhibition of diarrhea
I. Saline (2 ml/Kg)	0.5904 ± 1.08%	-
II. Loperamide (5 mg/Kg)	0.3350 ± 0.33%	43.26
III. CE (200 mg/Kg)	0.5140 ± 0.51%	12.94

Value are expressed as mean ±SEM (n=5). CE: combination of extracts Turmeric) rhizomes, Gall oak leaves, Guava leaves and Meniran herbs (1:1:1:1)

Table 2. Effect of combination of extracts Turmeric (*Curcuma domestica*) rhizomes, Gall oak (*Quercus lusitanica*) leaves, Guava (*Psidium guajava*) leaves and Meniran (*Phyllanthus niruri*) herbs on small intestinal transits in mice

Group Treatment (*p.o*)	Inhibition (%)
I. Saline (2 ml/Kg)	-
II. Loperamide (5 mg/Kg)	52.24
III. CE (200 mg/Kg)	96.08

CE: combination of extracts Turmeric rhizomes, Gall oak leaves, Guava leaves and Meniran herbs (1:1:1:1)

Table 3. Diameter inhibition zones, minimum inhibition concentration and minimum bactericidal concentration of combination of extracts Turmeric (*C. domestica*) rhizomes, Gall oak (*Q. lusitanica*) leaves, Guava (*P. guajava*) leaves and Meniran (*P. niruri*) herbs

Bakteri		Tetracycline (ppm)	CE (ppm)
Eschericia coli	DIZ	-	-
	MIC	2500	5000
	MBC	2500	5000
Salmonella thypii	DIZ	-	-
	MIC	625	1250
	MBC	625	1250
Staphylococcus aureus	DIZ	-	-
	MIC	2500	5000
	MBC	2500	5000

CE: CE: combination of extracts Turmeric rhizomes, Gall oak leaves, Guava leaves and Meniran herbs (1:1:1:1) DIZ: Diameter inhibition zones; MIC: minimum inhibition concentration; MBC: minimum bactericidal concentration; -: no activity at the concentration of the extracts tested.

flavonoids (Meniran (*P. niruri*) herbs, and curcuminoids (Turmeric (*C. domestica*) rhizomes) (Longanga *et al.* 2000). The antidiarrheal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretions (Rao et al. 1997) which are altered in this intestinal condition. In vitro and in vivo experiments have shown that flavonoids are able to inhibit the intestinal secretory response induced by prostaglandins E_2 (Sanchez et al. 1997). In addition, flavonoids present antioxidant properties which are presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism (Mora et al. 1990). Tannins present in antidiarrheal plants denature proteins in the intestinal mucosa by forming protein tannates which reduces secretion. The antibacterial activity of combination of ethanol extract of Turmeric (*C. domestica*) rhizomes, Gall oak (*Q. lusitanica*) leaves, Guava (*P. guajava*) leaves and Meniran (*P. niruri*) herbs against *S. aureus*, *E. coli* and *S.* Thypi was assessed by evaluating the presence of DIZ, MIC and MBC values. Results (Table 3) showed that the combination of extracts have weak antibacterial effects on *E. coli* and *S. aureus* but strongly inhibited the growth of *S.* Thypi. The absence of inhibition zone does not necessarily mean that compounds are inactive. For example, non-

polar compounds may not diffuse into the culture medium (Moreno et al. 2006).

CONCLUSION

The results revealed that the combination of plant extract with the doses of 200 mg/KgBW (1:1:1:1) reduced diarrhea in mice with relaxation of smooth muscle and showed weak antibacterial effects on *Eschericia coli* and *Staphylococcus aureus* but strongly inhibited the growth of *Salmonella* Thypi.

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