Screening for Antibacterial Properties of Some Plants and Chemical Antibiotic Against Two Isolates of *Escherichia coli* from Diarrhea Calves in Indonesia

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ABSTRACT

Plants used in factory medicine of Indonesian native people were collected. Ethanol extract and powder were prepared and evaluated in a test against two isolates of *Escherichia coli* from herbal plants mahkota dewa (*Phaleria macrocarpa*), daun sembukan (*Paederia foetida*), daun sirih/betel vine (*Piper betle*), kencur/greater galingale (*Kaempferia galangal*), garlic (*Allium sativum*) and jinten hitam (*Nigella sativa*). The results showed anti *E.coli* activity at 20% concentration with the most active plants with diameter of inhibition zones of 15 mm (*Allium sativum*) powder, 11 mm (*Piper betle*) extract and 14 mm (*Nigella sativa*) extract. Extract *Paederia foetida* and extract *Phaleria macrocarpa* had no inhibition effects. The two *E. coli* isolates were sensitive to chloramphenicol at 30 μg.

Key words: *antimicrobial, Escherichia coli, Allium sativum, piper betle, Nigella sativa, Paederia foetida, Phaleria macrocarpa, Kaempferia galangal*

INTRODUCTION

The spread of multi-drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Down the ages, spices have evoked interest as sources of natural products for their potential uses as alternative remedies to heal many infectious diseases (Parekh *et al*., 2005).

According to the reports of many researchers, antibacterial resistance is a worldwide growing-problem. Isolation of microbial agents less susceptible to regular antibiotics and recovery of resistant isolates during antibacterial therapy is increasing throughout the world. One of the measures to combat the increasing rate of resistance in long run, is to have continuous investigation for new, safe and effective antimicrobials as an alternative agents to substitute with no-effective ones. Natural resources, especially plants and microorganisms were the potent candidates for this rum. Usage of plants in curing illnesses has deep roots in man's history since plants are sources of many life-sustaining metabolites.

Colibacillosis incidences in cattle, pig and other farm animals were well documented in Indonesia. These bacterial incidences in young calves and piglets were reported in Bali (Hartaningsih and Hasan, 1985), Lampung (Suastama, 1983) and Central Java (Setiawan, *et al*., 1982). Piglet neonatal diarrhea associated with enterotoxigenic (ETEC) *Escherichia coli* was commonly observed in intensive piggeries in Bogor and Kapok areas. Here diarrhea occurred at the rates of 13 to 40 percents within the first two weeks of life. The associated mortality rates were from 12 to 30 percents (Supar *et al*., 1989). In turn, this young animal mortality contributed considerable losses to the national farm income.

As an effort to control diarrhea and other gastro-intestinal disorders, farmers regularly added antibiotics to farm animal feeds, especially in poultry and swine rations. In the long run, this practice may damage the animal health. Supar *et al*. (1990) proved that several *E. coli* isolates were resistant to commonly use antibiotics including Ampicillin, Streptomycin, Trimethoprim and Sulphamethoxazole. Further observation show that 100 *E. coli* strains were resistant to at least one antibiotic. The highest percentages being attained for resistance was to Penicillin, Tetracycline and Cephalothin (Carvalho *et al*., 1992).

Plants used in factory medicine of Indonesian native people were collected in this experiment. Ethanol extract and powder were prepared and evaluated in a test against two isolates of *Escherichia coli* from herbal plants; mahkota dewa (*Phaleria macrocarpa*), daun

sembukan (*Paederia foetida*), daun sirih/betel vine (*Piper betle*), kencur/greater galingale (*Kaempferia galangal*), garlic (*Allium sativum*) and jinten hitam (*Nigella sativa*).

MATERIALS AND METHODS

Materials

Methanol was used to make four concentrations of the plants drug (*Phaleria macrocarpa* extract, *Paederia foetida* extract, *Piper betle* extract, *Kaempferia galangal* extract, *Allium sativum* powder, and *Nigella sativa* extract) for this investigation. Mueller Hinton blood agar and broth media were used as growth media for the four bacterial isolates for this study. Additionally the blood agar media were also used as a purification control.

Isolates of *Escherichia coli* were collected from diarrhea calves in Indonesia by small farmers in Bogor, West Java. These specimens were later used for bacterial verification.

The obtained bacterial specimens were brought to BALITVET laboratory at Bogor. Here they were cultivated in the blood agar medium plates. The inoculated blood agar plates were incubated for 24 hours at 17^{o}C. The bacterial isolates grown in the blood agar plates were identified by employing Cowan and Steel methods (1973).

Extracting the Plants Drug

Dried the plants drug (*Phaleria macrocarpa* extract, *Paederia foetida* extract, *Piper betle* extract, *Kaempferia galangal* extract, *Allium sativum* powder, and *Nigella sativa* extract) were ground into powder. Methanol was then added to *Allium sativum* powder. To homogenize the mixture the liquefied the plants drug (*Phaleria macrocarpa* extract, *Paederia foetida* extract, *Piper betle* extract, *Kaempferia galangal* extract, *Allium sativum* powder, and *Nigella sativa* extract) was shaken for one hour. This agitation was necessary to accelerate the solution of the plants drug (*Phaleria macrocarpa* extract, *Paederia foetida* extract, *Piper betle* extract, *Kaempferia galangal* extract, *Allium sativum* powder, and *Nigella sativa* extract) active compounds in the methanol solvent. The mixture was kept for 24 hours. The liquefied of the plants drug was filtered by paper filter. The obtained methanol solution containing the plants drug active compounds, was poured into a Florentine tube. The tube was placed in a rotary evaporator to evaporate the methanol solvent at 40^{o}Celsius at 140-160 rpm and at 15-20 lbs of pressure.

Sterile aquadest was added to the obtained extracts to make four concentrations of the plants drug i.e. 20, 15, 10 and 5 percents. Then 15 micro liters of each concentration was dropped into a sterile paper disks. Each disk was laid on the MEU blood agar media that had been previously inoculated with each of the three bacterial isolates and were incubated for 24 hours at 37 ^{o}C.

The bacterial growth inhibition zones were observed and measured. The size of the growth inhibition zones indicates the effectiveness of the plants drug (*Phaleria macrocarpa* extract, *Paederia foetida* extract, *Piper betle* extract, *Kaempferia galangal* extract, *Allium sativum* powder, and *Nigella sativa* extract) in controlling the bacterial infection.

Experimental Design and Data Analysis

The first factor observed in this *in vitro* test was the type of the plant drug (*Phaleria macrocarpa* extract, *Paederia foetida* extract, *Piper betle* extract, *Kaempferia galangal* extract, *Allium sativum* powder, and *Nigella sativa* extract) on the inhibition growth of *Echerichia coli* isolates. There were four levels of this factor, i.e. *E. coli* isolates taken from diarhea calves. The second factor was the concentrations of the plant drug. This factor had five levels which was 20, 15, 10 and 5%. The observed dependent variable of this investigation was the determine differences among the means of the diameters of growth inhibition zones.

The Analysis of Variance (Anova) was used to analyze the data, the diameters of the bacterial growth inhibition zones. The Duncan Multiple Range Test (DMRT) procedure was used to determine differences among the means of the diameters of growth inhibition zones.

RESULTS AND DISCUSSION

The analysis of variances shows that the effects of plant drugs (*Phaleria macrocarpa* extract, *Paederia foetida* extract, *Piper betle* extract, *Kaempferia galangal* extract, *Allium sativum* powder, and *Nigella sativa* extract) growth inhibition zones were significant (Table 1). The results also showed that the greatest effects on bacterial growth inhibition were obtained when plant drugs of *Piper betle*, *Allium sativum* and *Nigella sativa* were used.

Table 1. The effect of plant drug 20% concentration on two *E.coli* growth inhibition

Plants drug at % concentration	Dried	Diameter of growth inhibition zone (mm)	
		E.coli (1)	*E.coli* (2)
Phaleria macrocarpa	extract	0	0
Paederia foetida	extract	0	0
Piper betle	extract	11	11
Kaempferia galangal	extract	0	0
Allium sativum	powder	15	15
Nigella sativa	extract	13.30	13.30

Table 2. The effects of type plant drugs of *Nigella sativa* extract, *Allium sativum* powder, and *Piper betle* extract on the bacterial growth inhibition zones

Plants drug	Diameter of growth inhibition zone (mm)	Level of significant*
Nigella sativa extract	8.83	b
Allium sativum powder	11.15	a
Piper betle extract	7.25	c

* Different alphabet code indicated a significant difference at (P<0.5).

The effects of plant drugs of *Piper betle*, *Allium sativa* and *Nigella sativa* on bacterial growth inhibition were confirmed by the results in Table 2. The highest effect was obtained from *Allium sativa* powder, then was followed by *Nigella sativa* and *Piper betle* extracts (P<0.05).

Table 3 demonstrates results of effects of different concentrations of plant drugs applied on growth inhibition zones. The results indicate that the increase in concentrations from 5 up to 20% produced greater effects in growth inhibition zones. The greatest growth inhibition zone was obtained at the greatest concentration, i.e. 20%.

Table 3. The main effect of the plant drug concentration increase on the bacterial growth inhibition

Plants drug concentration	Diameter of bacterial growth inhibition zone (mm)	Level of significant*
20.00	13.11	a
15.00	10.44	b
10.00	7.67	c
5.00	5.11	d

* Different alphabet codes indicate a significant difference at (P<0.5).

Table 4 indicates the combine effects of concentration and the three plant drugs (*Piper betle* extract, *Allium sativum* powder, and *Nigella sativa* extract) on *E. coli* growth inhibition zones. The results demonstrated that *Allium sativum* powder produced the largest growth inhibition zones which differed significantly from those obtained by *Nigella sativa* and *Piper betle* extracts at all concentrations. The largest growth inhibition zones were obtained by using *Allium sativum* powder at 20% concentration.

Table 4. Effects of combination between extract concentration and types of several plant drugs on *E.coli* growth inhibition zones

Extract concentration (%)	Type of plant drugs	Diameter of growth inhibition zones (mm)	Significant level
20 %	*Nigella sativa* extract	14.3	b
	Allium sativum powder	15.0	a
	Piper betle extract	11.0	c d
15 %	*Nigella sativa* extract	10.0	d
	Allium sativum powder	12.3	b c
	Piper betle extract	9.3	e
10 %	*Nigella sativa* extract	7.0	f g
	Allium sativum powder	10.0	d
	Piper betle extract	6.0	g
5 %	*Nigella sativa* extract	5.0	h
	Allium sativum powder	7.3	f
	Piper betle extract	3.3	i

*Different alphabet code indicated a significant difference at P<0,5 DMRT.

CONCLUSIONS

The plant drugs *Phaleria macrocarpa* extract, *Paederia foetida* extract, *Piper betle* extract, *Kaempferia galangal* extract, *Allium sativum* powder, and *Nigella sativa* extract had bactericidal effect on *E. coli.*

The higher the concentration of the plant drugs (*Phaleria macrocarpa* extract, *Paederia foetida* extract, *Piper betle* extract, *Kaempferia galangal* extract, *Allium sativum* powder, and *Nigella sativa* extract), the larger the diameter of the bacterial growth inhibition zones obtained of the three plant drugs tested, *E. coli* growth was the most affected by *Allium sativum* powder at all concentrations.

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