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The *Escherichia coli* Growth Inhibition Activity of Some Fermented Medicinal Plant Leaf Extract from the Karo Highland, North Sumatra

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ABSTRACT

A lot of traditional medicinal plant has antibacterial activities. Most of these plants are freshly chewed or grounded and used directly to treat infectious bacterial diseases. However, some practices employ a traditionally spontaneous fermentation on boiled extracted leaf, root or other parts of the plant. This work reports a laboratory stimulated spontaneous fermentation of leaf extracts from selected medicinal plants collected from the Karo Higland. The spontaneous fermentation was stimulated to be carried out by the *Acetobacter xylinum* and *Saccharomyces cerevisiae*. The anti-infectious agent activity was assayed on the *Escherichia coli* growth inhibition. A complementary non fermented leaf extract was also made and assayed as a comparative measure. Indeed, the fermented leaf extract of bitter bush (*Eupatorium pallescens*), cacao (*Theobroma cacao)*, avocado (*Persea gratissima),* passion fruit (*Passiflora edulis)*, cassava (*Cassava utilissima)*, diamond flower (*Hedyotis corymbosa*), periwinkle (*Catharanthus roseus*), and gandarussa (*Justicia gendarussa*) have relatively higher anti-*E. coli* activity than those of non fermented ones. However, there were no anti-*E. coli* activity was detected in both fermented and non fermented leaf extract of the guava *(Psidium guajava)* and common betel (*Piper nigrum).*
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Key words: medicinal plant, anti-bacteria, *Escherichia coli*, fermentation, *Acetobacter xylinum*, *Saccharomyces cerevisiae*.

INTRODUCTION

Indonesia is one of the two mega-centers of biodiversity, alongside Brazil, with around 140 million hectares of rain forest. Therefore nature is deeply rooted in the life of people socially, economically and culturally. The flora diversity has been taken advantages for traditional remedies since ancient time (Ministry of Trade, 2006). It has been proved that many plants have healing and disease prevention properties as well as maintaining health or curing ailments. The plants known or believed having such pharmacological effects were called medicinal plants (Maiti, 2007). Medicinal plants are categorized into three groups: traditional medicinal plants are plants which known or believed having medicinal properties and have been used as traditional medicines; modern medicinal plants are plants which proved scientifically to produce medicinal lead compound and its application can be verified medically, and; potential medicinal plants are plants which have been proved scientifically to produce medicinal bioactive compound, nevertheless medically invalidated or its application as traditional medicines are difficult to trace (Zuhud et al., 1994).

Most of traditionally Indonesia people have known and applied medicinal plants as one of the effort to deal with healthy issue before modern drugs were developed. At present days, they are still used as one of the alternative medication, and so-called traditional medicine. Traditional medicine may be seen as a product of the twofold wealth of Indonesia: its biodiversity and its cultural diversity. About 2500 medicinal plant species are presently used as traditional medicines. It relied on bioactive compound produced by the plants (Ministry of Trade, 2006). The diversity of bioactive compound produced by the plants rise efforts to further develop of drugs for many diseases, such as antibacterial agent, antifungal agent, antiparasites, anticancer, antivirus, etc (Slikkerveer, 2006).

Most of the plants live in volcanic areas rich in selenium and sulphur, such as the Karo highlands may bear selenium based active compounds. In fact, selenium base bioactive compounds are one of the important assets from Indonesia microbial diversity. Previous studies have discovered spectrum of biological functions of those bioactive compound, such as antioxidant of lipid membrane peroxidation which play an important role in binding lipid radical in atherosclerosis prevention (Bulger and Maier, 2001); lowering cholesterol, anti cancer (Arteel and Sies, 2001; Dong et al., 2002), controlling hormonal balance, reproduction and normal growth of baby and

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children (Maehira et al., 2002), slowing aging, increasing fertility (Ellis et al., 2004; Kiefer, 2004) and anti microbes, including antibacteria which have significant importance in immune systems (Bulger and Maier 2001; Stanner 2001).

Human body cannot synthesize selenoprotein. Therefore selenium intake depends on nutrient consumption. One way to fulfill the need of selenoprotein is bio-extraction of selenium from medicinal plant leaves and selenoprotein synthesis using a mixed acid fermentation of *Acetobacter xylinum* and *Saccharomyces cerevisiae*. This system will decrease pH to very low state in order to hydrolyze selenium that bind with methionine and cysteine to provide amino acid binding selenium that available to be absorbed and synthesized into selenoprotein by the microorganisms (Puspitasari, 2001).

This preliminary research was conducted to reveal the antibacterial properties as one of the selenium base bioactive compound activities produced in a mixed acid fermentation of selected medicinal plant leaves. The extract was subjected to assay on the growth inhibition of *Escherichia coli* as causal agent of diarrhea.

MATERIAL AND METHODS

Bacterial culture. The microorganisms that were employed were *A. xylinum* and *S. cerevisiae*. These bacteria and yeast isolates were isolated from Indonesian traditional fermented food, i.e. from the Karo Highland, North Sumatra by Genetics Laboratory, Department of Microbiology, Research Center for Biology, LIPI Cibinong-Bogor, West Java. The *E. coli* JM109 was used in the antibacterial assay.

Preparation of assay bacteria. The assay bacteria, *E. coli*, was streaked on Nutrient Agar (NA) media that prepared from 28 g NA (ready to use) in 1000 mL distilled water, then incubated at 37^oC for 3 days. Two loops of good growth *E. coli* were inoculated in 100 mL Nutrient Broth (8 g in 1000 mL distilled water), then incubated at 37^oC for 24 hours for further used.

Medicinal plants material. The medicinal plants were selected based on their etnobotanical uses in infectious disease prevention and therapy as revealed by survey and interview some saman and people in the Karo Highland areas. Those plants were bitter bush (*Eupatorium odoratum* L), cacao (*Theobroma cacao* L), avocado (*Persia gratissima* Gaerth), guava (*Psidium guajava* L), betel (*Piper betle* L), passion fruit (*Passiflora edulis* Sims), cassava (*Manihot utilissima* Pohl), diamond flower (*Hedyotis corymbosa* [L] Lamk), periwinkle (*Catharanthus roseus* [L] G.Don), and gandarussa (*Justicia gendarussa* Burn). The plant leaves were grounded and then oven dried at 40^oC for two days.

Preparing starter *A. xylinum-S. cerevisiae.* Starter was prepared from pineapples juice added with distilled water at composition 30:70 (v/v) and sugar at 5% (w/v) of total volume. The mixture was aliquoted into several little jars then covered with cheese cloth, plastics, and tied with rubber, then autoclaved at 121^oC, 15 psi for 15 minutes. After cooling, media were inoculated with *A. xylinum-S. cerevisiae* (10% total volume), then incubated at room temperature for 3-4 days to grow nata layer, for further inoculation to the leaf extract of medicinal plants.

Leaf extract fermentation. One half gram of dried leaves was added with 10 g sucrose and 50 mL distilled water, then covered with cheese cloth, paper, plastics, and tied with rubber, then autoclaved at 121^oC, 15 psi for 15 minutes. Each sample consisted of 2 repeats and 1 control. All samples but controls were inoculated with 5 mL starter *A. xylinum-S. cerevisiae*. Fermentation process was performed for 9 days.

Fermented leaf suspension preparation. After 9 days fermentation, pH of extracts was measured. As much as 1.5 mL extract was centrifuged at 3.000 rpm for 10 minutes. Supernathan was used for anti *E. coli* test.

Assay of anti-*E. coli* activity of leaf extract. The *E. coli* were swabbed on the NA agar surface using soft tip to evenly distributed and dried. Paper discs were dipped and saturated in each leaf extract for 5 minutes. It was then put onto the surface of the media inoculated with *E. coli*, then incubated at 30^oC for 3 days to allow the bacterial growth. Anti-*E. coli* activity of the extract were then determined by measuring the respective zone of inhibition.

RESULTS AND DISCUSSION

The mixed acid fermented leaf extract suspension had lower pH that that of the non fermented extract suspension (Table 1). It probably due to sugar fermentation by the *S. cerevisiae* producing ethanol and carbondioxide (CO_2). The ethanol was then oxidized by *A. xylinum* to form acetic acid that in turn acidified the extracts suspension. The acid facilitated the selenium extraction and selenoprotein biosynthesis by the mixed acid fermentative microorganisms (Puspitasari, 2001).

Tabel 1. pH of control and the mean of treatment (fermented leaf extracts)

Plants	Mean of pH of treatment	pH of control
Bitter bush	2,992	5,705
Cacao	3,103	6,055
Avocado	2,868	6,083
Guava	3,106	5,000
Betel	2,976	6,450
Passion fruit	3,021	5,853
Cassava	2,902	5,318
Diamond flower	3,101	5,364
Periwinkle	3,255	5,598
Gandarussa	3,544	8,081

Table 2 shows anti-*E. coli* growth of the selected fermented leaf suspension. This might indicates that some fermented leaf extracts contained active compound of selenium. Selenium is bioactive compound that has antibacterial activity (Stanner 2001).

Tabel 2. Diameter of inhibition zone of fermented leaf extracts and control against assay bacteria *E. coli.*

Plants	Diameter of inhibition zone (cm)	
	Fermented leaf extract*	Control (without fermentation)
Bitter bush	2,53	2,90
Cacao	1,60	1,36
Avocado	1,22	-
Guava	-	-
Betel	-	-
Passion fruit	1,53	-
Cassava	1,51	-
Diamond flower	1,84	-
Periwinkle	1,58	-
Gandarussa	1,51	1,65

Note:-not detected, * fermented by *A. xylinum* and *S. cerevisiae*

The medicinal plants having high potential anti-*E. coli* were bitter bush, cacao, avocado, passion fruit, cassava, diamond flower, periwinkle, and gandarussa. Their potency could be predicted from their leaf extracts activity to inhibit the growth of *E. coli* as indicated by the clear zone of no observed growth around the paper discs contained leaf extracts. In this study, leaf extract of passion fruit exhibited anti-*E. coli* activity. This result confirmed Ripa's statement (2009) that such activity of chloroform extract of passion fruit leaf was exhibited. Probably the anti-*E. coli* activity of diamond flower and periwinkle is the first report since no available report yet. Both plants were known well for their activity against cancer cells (Sadasivan et al., 2006; Sudarsono, 1999; Taha et al., 2008). There is no available report yet also on the anti-*E. coli* activity of the gandarussa (Panthi and Chaudhary, 2006).

However, there is a phenomenon that the anti-*E. coli* was detected after fermentation in some plant such as avocado, passion fruit, cassava, diamond flower, and periwinkle. That means the bioactive compounds need to be activated.

No active compound activation is required in the case of plants bitter bush, cacao, and gandarussa. It is probably the nature of the active compound that could be extracted well with hot water and acidification during the fermentation did not play any comparative role. Table 2. showed that potential anti *E. coli* of unfermented leaf extracts of bitter bush, cacao, and gandarussa were relatively higher than fermented ones. One of the explanations is that selenium concentration in the beginning of fermentation process might be used in the microbial metabolisms such as selenoprotein biosinthesis that transform the anti-*E. coli* into other functional molecules. In this case, instead of using fermented leaf extracts, direct use of unfermented ones to treat infectious disease caused by *E. coli* thought to be more effective, because it showed higher anti-*E. coli* activity. This result confirmed Heyne's statement (1987) that bitter bush leaves have been long ago known and applied as traditional medicine to treat chronically diarrhea. In application, those leaves were used directly without fermentation process. Unfortunately, the potential anti-*E. coli* of cacao and gandarussa leaves has not been reported. Nevertheless, gandarussa is used to cure stomachache in Aceh (Ministry of Trade, 2006).

Leaf extracts of guava and betel showed no anti-*E. coli* activity. Therefore hot water extract of betel and guava leaves could not be used as anti-*E. coli* medicine. However Syamsuhidayat et al. (1991) reported that guava and betel leaves have been long ago known and used as folklore medicine to cure diarrhea caused by *E. coli*. It suggests that the anti-diarrhea activity of the guava leaf is not anti-*E. coli* in term of growth inhibition, probably the prevention of water excretion or inactivation of the causative microbial toxins (Hoque, 2007). On the other hand, anti-*E. coli* activity was exhibited by ethanol extract of betel leaf (Arambewala et al., 2005).

In addition to leaves of bitter bush and guava which were long ago believed as anti diarrhea in traditional medicine (Morton, 1987; Heyne, 1987), the anti-*E. coli* activity of leaves of cacao, avocado, passion fruit, cassava, diamond flower, periwinkle, and gandarussa may enrich the etnobotanical information since those plants were not commonly employed as anti diarrhea or anti-*E. coli*.

Based on the diameter of inhibition zone of the anti-*E. coli* of fermented leaf extracts, it could be list from the highest to the lowest were bitter bush, diamond flower, cacao, periwinkle, passion fruit; followed by gandarussa and cassava which have same activity; and avocado, respectively. The highest anti-*E. coli* of unfermented leaf extracts was bitter bush, followed by gandarussa, and cacao, respectively. Meanwhile, the leaf extracts of guava and betel exhibited no anti-*E. coli* activity.

CONCLUSSION

The fermentation facilitated the leaf extracts of diamond flower, periwinkle, passion fruit, cassava, and avocado to demonstrate anti-*E. coli* activity. The leaf extracts of bitter bush, gandarussa, and cacao exhibited anti-*E. coli* activity with or without fermentation, therefore they could be used directly as anti-*E. coli* medicine. On the other hand, the leaf extracts of guava and betel exhibit no antibacterial activity with or without fermentation process.

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