



Antibacterial Properties of the Extracts from Some Zingiberaceous Species in Thailand against Bacteria Causing Diarrhea and Food Poisoning in Human

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ARTICLE INFO

Article history:
Received 04 May 2015
Received in revised form
18 June 2015
Accepted 30 June 2015
Available online
01 July 2015

Keywords:
zingiberaceous plant
extracts;
Zingiberaceae;
Ethanol extract;
Aerobic;
Anaerobic;

ABSTRACT

Many of Zingiberaceous plants play important roles in Thai society more than hundred years ago. For example, they are used as herbal medicines for disorders related to digestive tract. Even though the pharmacology and chemical compositions of these plants were reported, but there are only a few reports on antibacterial activities. This study intend to screen for antibacterial property of the ethanolic extracts from some Zingiberaceae species i.e. *Zingiber montanum*, *Z. ottensii*, *Z. officinale*, *Z. zerumbet*, *Zingiber 'Phlai Chomphu'*, *Alpinia galanga*, *Boesenbergia rotunda*, and *Kaempferia parviflora*. The ethanolic extracts were tested with bacteria which are the common causes of diarrhea and food poisoning in humans, *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, and *Mycobacterium smegmatis*.

The test, OD was measured under both aerobic and anaerobic conditions. It found that under aerobic condition the extracts of *Z. officinale* and *Zingiber 'Phlai Chomphu'* showed the effect against *L. monocytogenes* while the extracts from *Zingiber 'Phlai Chomphu'*, *B. rotunda* and *K. parviflora* showed the effect against *M. smegmatis*. For anaerobic condition, the extracts of *Z. officinale*, *Zingiber 'Phlai Chomphu'* and *A. galanga* showed the effect against *S. typhimurium* whereas the extracts of *K. parviflora* showed the effect against *S. aureus*. The extracts of *Z. montanum*, *Z. ottensii* and *Z. zerumbet* showed no antibacterial property while those of *Zingiber 'Phlai Chomphu'* and *A. galanga* showed the strongest antimicrobial property.

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1. Introduction

Plants of the family Zingiberaceae comprises about 1,400 species worldwide. About 1,000 zingiberaceous species are found in tropical Asia, 300 species have been recorded in Thailand (Larsen and Saksuwan Larsen, 2006). They are important natural resources that provide many useful products for food, spices, medicines, dyes, perfumes and aesthetics. The main zingiberaceous genera are *Alpinia*, *Amomum*, *Curcuma*, *Elettaria*, *Hedychium*, *Kaempferia* and *Zingiber*. Numerous chemical compounds in zingiberaceous plants have been reported (Sirirugsa, 1999; Norajit et al., 2007; Tripathi et al., 2013).

The gastrointestinal problems, including diarrhea and food poisoning, are common in many tropical countries. The bacterium that causes these problems is *Enterococcus faecalis* which is gram positive. It is also a major cause of urinary tract infections, bacteremia, infective endocarditis, opportunistic infections, meningitis, and nosocomial bloodstream infections (Paulen et al., 2003; Massier et al., 2012).

Escherichia coli is a gram negative bacterium. *E. coli* subtypes and may vary widely contaminated in ecosystem. Contaminated both water and then to contaminate the food. In human it is a common inhabitant of the gastrointestinal tract. It can also cause various intestinal which can develop to extra-intestinal diseases (Nelson et al., 2008; Jay, 2000).

Listeria monocytogenes, a gram positive rod, causes listeriosis in human, with common manifestation in gastroenteritis, septicemia, meningitis, encephalitis, central nervous system infections, blood-brain, brain abscesses, corneal ulcer, pneumonia, and in a perinatal infection. Pregnant women usually get infected but are not always cervical infections, which may results in spontaneous abortion or stillbirth, placental barriers or mother-to-child infections. (Gray and Killinger, 1966; Holland et al., 1987; Farber and Peterkin, 1991; Dussurget et al., 2004)

Salmonella typhimurium, a gram negative bacterium that often contaminates poultry products, causes foodborne salmonellosis. It is also a major cause of human gastroenteritis. When an infection occurs, often with an invasion into the bloodstream (bacteremia), it may cause fever and chills, body aches, loss of appetite, and weight loss. In children, often infect in brain and be meningitis and may be pneumonia, osteomyelitis, septic arthritis, pericarditis, pyelitis, peritonitis, otitis media, cholecystitis, endophthalmitis, cutaneous abscess (Horton et al., 2000; Kerdpanich, 2006)

Staphylococcus aureus is a gram positive bacterium. It produces poison/toxin that causes illness, several disease syndromes in human and animals such as food poisoning or food

intoxication and foodborne gastroenteritis. A symptom are nausea, vomiting, abdominal cramps (which are usually quite severe), diarrhea, sweating, headache, prostration, and sometimes a fall in body temperature. It is the most common cause of skin and soft-tissue infections, as well as of invasive infections acquired in hospitals and bloodstream infection after cardiac surgery. (Jay, 2000; Carrier *et al.*, 2002; Fridkin *et al.*, 2005; Oonmetta-aree *et al.*, 2006; Stehulak, 2011)

Mycobacterium smegmatis, a gram positive bacterium, is considered a non-pathogenic microorganism. It does not cause disseminated disease even in immuno-suppressed individuals. In some very rare cases, it may cause disease because it is related to immunosuppression. It grows at a relatively high rate in many defined and nutrient-restricted media. As a result, *M. smegmatis* has been used as an appropriate model for studying the pathogenesis of tuberculosis. (Reyrat and Kahn, 2001; Best and Best, 2009; Jin *et al.*, 2010; Cordone *et al.*, 2011)

The most commonly claimed efficacies of the zingiberaceous plants in Thai traditional medicine are for treatment of various symptoms related to digestive system such as stomachache, diarrhea, flatulence, and stomach ulcer. These plants include *Alpinia galanga*, *Boesenbergia rotunda*, *Curcuma longa*, *Kaempferia parviflora*, and *Zingiber officinale*. Other distinct medicinal properties such as aphrodisiac and diuretic are also mentioned in *B. rotunda*, anti-motion sickness in *Z. officinale*, and counterpain in *Zingiber montanum* (Farnsworth and Bunyaphatsara, 1992).

From previous studies, *Z. officinale*, *Z. montanum*, *Z. zerumbet* are Zingiberaceous Plants which is popular to study. The plants in Zingiberaceae are diversified and they have been given interests within the study of pharmacology and elements seriously. However studies related to the antibacterial activity is minimal. Therefore, this study about zingiberaceous plants screening for test antibacterial activity, it is certainly important and interesting to be exploited further.

2. Materials and Methods

2.1 Plant materials

Mature, 8-month old rhizomes of *Z. montanum*, *Z. ottensii*, *Z. officinale*, *Z. zerumbet*, *Zingiber* ‘Phlai Chomphu’, *A. galanga*, *B. rotunda* and *K. parviflora* were purchased from a local market in Bangkok, Thailand. They were washed, air dried at room temperature, sliced and freeze dried. The freeze dried - sliced rhizome were ground into fine powder with mortar and pestle and stored in Ziploc plastic bags to protect them from sunlight and moisture at room

temperature (26.5°C).

2.2 Extraction

One gram of powdered samples was mixed with 10 ml 95% ethanol in glass vial and shake well with vortex mixer and the extract left at room temperature overnight. Each sample was centrifuged at 1,000 rpm for 3 min at 20°C to settling. The 100 µl suspension was filtered through syringe filter with 0.2 µm membrane, and the sample was kept at -20°C until analyzed.

2.3 Antibacterial activities

Four gram positive and two gram negative bacteria were used for the antibacterial activity assay. *E. coli* (O157:H7), *S. typhimurium* (LT2), *S. aureus* (Mu50), *L. monocytogenes* (EDGE), *E. faecalis* and *M. smegmatis* (LM143) were obtained from the public culture collection (ATCC or DSMz). Before each use, frozen stock cultures of bacteria were thawed and grown in a chemically defined medium (CDM) (Jensen and Hammer, 1993) at 37°C for 20 h under aerobic and anaerobic conditions (6% hydrogen, 20% CO₂ and balanced with nitrogen). The cultures were inoculated using overnight culture of each bacterium at OD_{600nm} = 0.1 in 150 µl of CDM with (2% of the zingiberaceous extract) or without zingiberaceous extract and performed in 96 wells microplate. The kinetic growths were performed in CDM at 37°C for 30 h using microplate reader, and 72 h for *M. smegmatis* in aerobic (Beckman Coulter DTX 800/880 microplate reader) and anaerobic conditions (Biotek ELx808 microplate reader). Each experiment was conducted in three replicates.

3. Results and Discussion

The bacterial growth was evaluated in the presence and absence of zingiberaceous ethanolic extracts. *Z. montanum*, *Z. ottensii* and *Z. zerumbet* extracts showed no antibacterial activity to all of the tested bacteria.

No tested zingiberaceous extract have shown antimicrobial activity against *E. coli* (O157:H7) in aerobic or anaerobic condition. These results agreed with similar observations by Indu *et al.* (2006) which reported that ginger extract did not show any antibacterial activity against all other serogroups of *E. coli*. These results are different according to the observations of Debbarma *et al.* (2012) who had reported the ginger essential oils have antibacterial activity on *E. coli*. The serogroups and the zingiberaceous extracts seemed to have an impact on the antimicrobial activity (Habsah *et al.*, 2000; Indu *et al.*, 2006; Chen *et al.*, 2008).

In aerobic condition, *Z. officinale* and *Z. 'Phlai Chomphu'* inhibited *L. monocytogenes*. Indeed, this condition, the growths of *L. monocytogenes* increased to OD_{600nm} = 0.4 during the first 15h then decreased to OD_{600nm} = 0.2 at the final point in control condition. In the presence

of *Z. officinale* and *Z. 'Phlai Chomphu'* extracts, *L. monocytogenes* was unable to grow (Fig. 1). These results are in contradiction with others studies which showed *Z. officinale* extract with no inhibitory effect on *L. monocytogenes* growth by Thongson *et al.* (2005) and Indu *et al.* (2006). However, the extraction methods (50% ethanol or water) were different from our extraction method (95% ethanol).

The growth of *M. smegmatis* gradually increased from $OD_{600nm} = 0.1$ to 0.3 during 72 h in control condition. In the presence of *Zingiber 'Phlai Chomphu'*, *B. rotunda* and *K. parviflora* extracts, the final *M. smegmatis* growths decreased 0.15 OD_{600nm} units when compared with ethanol control. We observed strong inhibition on *M. smegmatis* growth in the presence of *A. galanga* extract. Several studies showed that mycobacterium species can be strongly inhibited by 10-gingerol compound extracted from *Z. officinale* (Hiserodt *et al.*, 1998; Newton *et al.*, 2000). In our study, *Z. officinale* did not inhibit *M. smegmatis*. This compound was probably be in low concentration in our extract. However, *Z. 'Phlai Chomphu'*, *B. rotunda*, *K. parviflora*, and *A. galanga* showed an antimycobacterial activity suggesting the presence of 10-gingerol compounds sufficient to inhibit *M. smegmatis* (Fig. 2).

In anaerobic condition, the growth of *S. typhimurium* increased to $OD_{600nm} = 0.4$ during 10 h and then became stable at $OD_{600nm} = 0.35$ until the final point. Only in anaerobic condition, *Z. officinale*, *Z. 'Phlai Chomphu'* and *A. galanga* inhibited *S. typhimurium* (Fig. 3). The results are in agreement with many previous reports indicated moderate or no antibacterial activity against *S. typhi* or *S. typhimurium* (Indu *et al.*, 2006; Debbarma *et al.*, 2012)

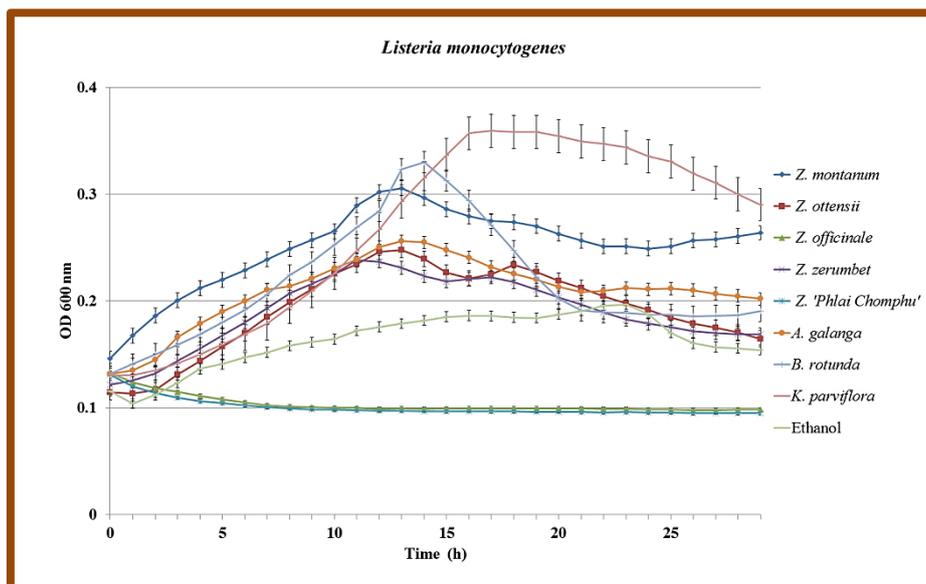


Figure 1: The growth of *Listeria monocytogenes* tested with extract of some *Zingiberaceae* in aerobic condition.

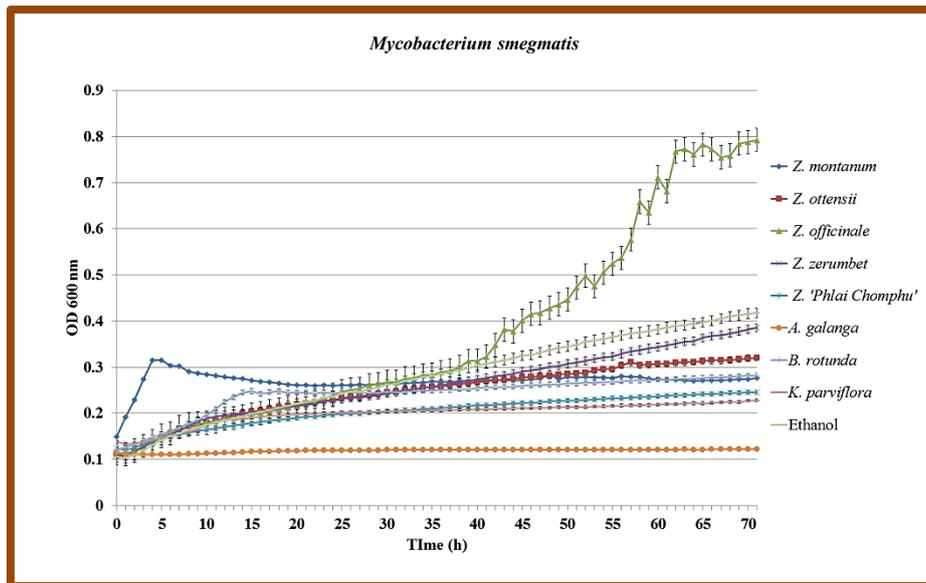


Figure 2: The growth of *Mycobacterium smegmatis* tested with extract of some Zingiberaceae in aerobic condition.

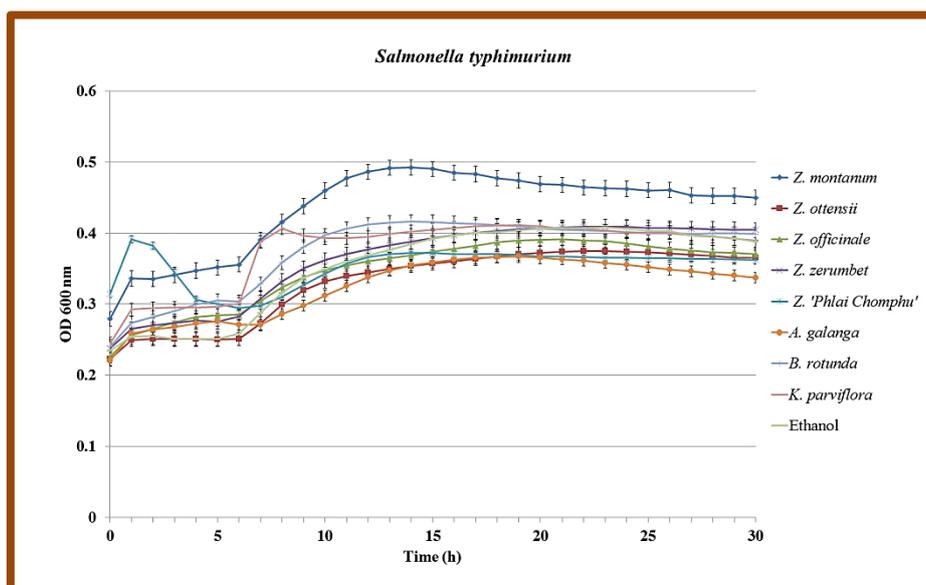


Figure 3: The growth of *Salmonella typhimurium* tested with extract of some Zingiberaceae in anaerobic condition.

Regarding *E. faecalis*, the bacterial growth increased to $OD_{600nm} = 0.5$ during 10 hour, and clearly decreased after 11 hour in the presence of *K. parviflora* extract in anaerobic condition (Fig. 4). For this bacterium only *Z. officinale* was tested in aerobic condition in several studies. The results showed no antibacterial activity against *E. faecalis* (Hammer *et al.*, 1999).

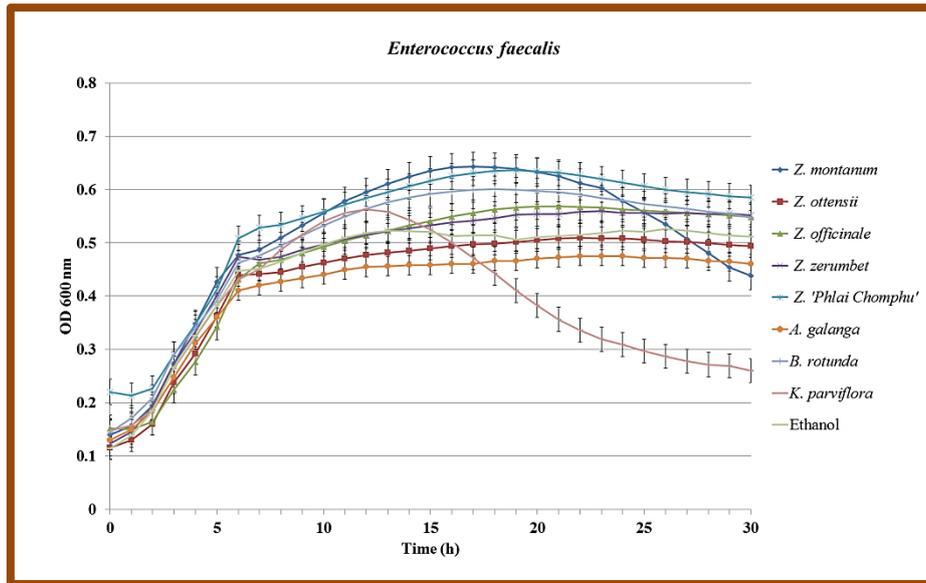


Figure 4: The growth of *Enterococcus faecalis* tested with extract of some Zingiberaceae in anaerobic condition.

A. galanga inhibited *S. aureus*. In the first 10 h, the growth of *S. aureus* in all treatments was lower than control ethanol then increased to OD_{600nm} = 0.4 and became stable until the final point except *A. galanga* extract which showed strong inhibitory effect on the growth of bacteria. Only *A. galanga* inhibited *S. aureus* (Fig. 5). Habsah *et al.* (2000) reported the activity of several zingiberaceous extracts against *S. aureus* but the extraction was done by using chloroform or methanol. However, our results are in agreement with Chen *et al.* (2008) who showed the various extracts have different activity against *S. aureus*

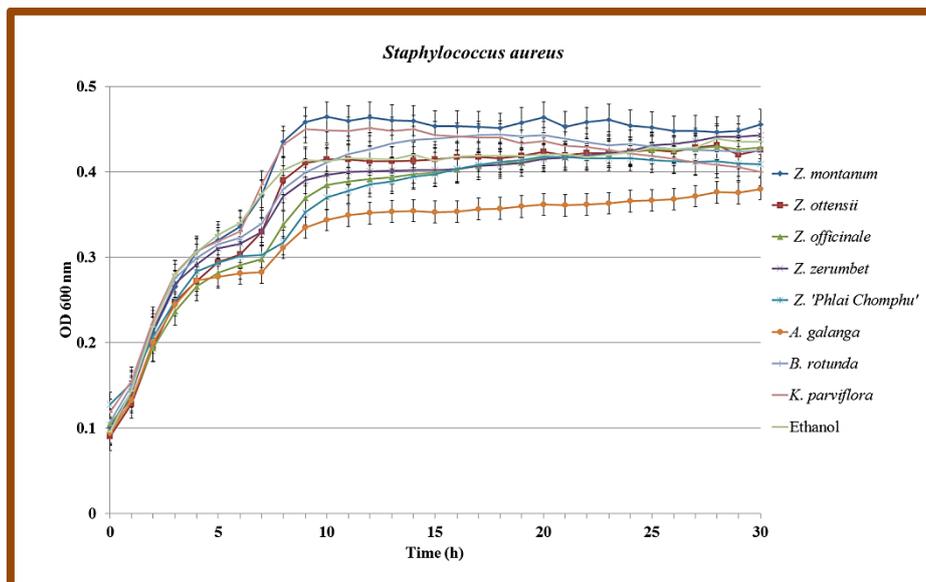


Figure 5: The growth of *Staphylococcus aureus* tested with extract of some Zingiberaceae in anaerobic condition.

4. Conclusions

Under aerobic condition, *Z. officinale* could inhibit *L. monocytogenes*, while *Z. 'Phlai Chomphu'* could inhibit both *L. monocytogenes* and *M. smegmatis*. *A. galanga*, *B. rotunda* and *K. parviflora* could inhibit *M. smegmatis*.

Under anaerobic condition, *Z. officinale* and *Z. 'Phlai Chomphu'* could inhibit *S. typhimurium* while *A. galanga* could inhibit both *S. typhimurium* and *S. aureus*, and *K. parviflora* could inhibit *E. faecalis*.

This study indicated and confirmed that the ginger species showed various antimicrobial activities. Four ginger ethanolic extracts, *K. parviflora*, *Zingiber 'Phlai Chomphu'*, *A. galanga*, and *B. rotunda*, are able to inhibit *M. smegmatis*.

K. parviflora showed an antimicrobial activity against gram positive bacteria while *Z. officinale*, *Z. 'Phlai Chomphu'* and *A. galanga* are able to inhibit both gram positive and gram negative bacteria whereas *Zingiber 'Phlai Chomphu'* and *A. galanga* showed strongest antimicrobial activities.

5. Acknowledgements

We thank the Department of Horticulture, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand for financial support as well as the Population Health and Reproduction Department, School of Veterinary Medicine, University of California Davis for technical assistance. The first author is grateful to Prof. Bart. C. Weimer for his kind facilitations during the author's stay at the University of California Davis.

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