

## **Inhibitory Effects of Selected Thai Spices and Medicinal Plants on *Escherichia coli* O157 : H 7 and *Yersinia enterocolitica***

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### **ABSTRACT**

The effects of spices and medicinal plants were tested for their inhibitory effects on newly foodborne pathogens namely *Escherichia coli* O157 : H 7 and *Yersinia enterocolitica* by the well assay technique. Crude extracts of cloves (*Eugenia caryophyllata* Thunb.) and cinnamon (*Cinnamomum zeylanicum* Nees.) revealed high antibacterial activity on both organisms. The betle leaf (*Piper betle*) showed the highest activity on *E. coli* O157 : H 7, whereas the pomegranate rind (*Punica granatum* Linn.) exhibited the highest antibacterial action on *Y. enterocolitica*. The effects of pH and temperature on antibacterial activity of plant extracts were explored. The antibacterial activity of plant extracts on the test organisms at selected pH values indicated that it was more active at low pH than at high pH. At low temperatures (4 and 10°C) the plant extracts have less activity than at high temperatures (25, 35 and 45°C).

**Key words:** spices, medicinal plants, antibacterial activity, *Escherichia coli* O157 : H7, *Yersinia enterocolitica*

### **INTRODUCTION**

In recent years there has been a dramatic increase in the number of reported cases of foodborne illness. *Escherichia coli* O157 : H 7 is a highly virulent bacterium. Its transmission to human has recently been epidemiologically associated with the consumption of contaminated foods. This pathogen is an etiological agent of hemorrhagic colitis and hemolytic uremic syndrome in humans. Its reported mortality rates was as high as 31% (Sack, 1987). Although outbreaks of food-associated *E. coli* O157 : H 7 illness have been associated with the consumption of undercooked ground beef, this bacterium has been isolated from

drinking water, apple cider, mayonnaise and mayonnaise-based sauces (Doyle *et al.*, 1997).

*Yersinia enterocolitica* is an important foodborne pathogen because of its ability to grow at refrigeration temperatures. *Yersiniae* readily withstand freezing and can survive in frozen foods for extended periods even after repeated freezing and thawing (Toora *et al.*, 1992). Foodborne outbreaks due to *Y. enterocolitica* have been related to raw milk, powdered milk, chocolate – flavored milk, tofu, bean sprouts, pork, cheese and pork chitterlings (Robins – Browne, 1997).

Consequently, there is considerable interest in ways to stop this upward trend and reduce the incidence of food poisoning. One area of research

is the development of new and improved methods of food preservation. Due to negative consumer perceptions of artificial preservatives, attention is shifting towards alternatives that the consumers perceive as natural, an in particular, spices and medicinal plants or herbs. Several spices and medicinal plants have been known to possess antimicrobial activities (Evert Ting and Deibel, 1992; Stonsaovapak *et al.*, 1995; Smith – Palmer *et al.*, 1998).

In searching for natural antimicrobial products, the present study provides data on antibacterial activity of various spices and medicinal plants that are available in Thailand against foodborne pathogens namely *E. coli* O157 : H7 and *Y. enterocolitica*.

## MATERIALS AND METHODS

### Maintenance and preparation of cultures

Cultures of *E. coli* O157 : H7 ATCC 43889 and *Y. enterocolitica* ATCC 23715 were obtained from the National Institute of Health, Ministry of Public Health, Bangkok, Thailand. Cultures were grown in Tryptic Soy Agar, TSA (Merck, Darmstadt) at 37°C and maintained on TSA at 4°C. Before preparing inoculum for the test media, cultures were activated by two successive transfers in Tryptic Soy Broth (TSB) at 37°C. Inoculum population was determined by serially diluting the suspension with phosphate buffer, pour plating with TSA and incubating at 37°C for 48 hr before counting colonies.

### Spices and medicinal plants extraction

Spices and medicinal plants used in this experiment are shown in Table 1. They were dried and ground before extracting with 95% ethyl alcohol for 48 hr. The volumes were adjusted to obtain the concentration of 100 mg/ml. The aqueous extract was sterilized by millipore membrane (0.45  $\mu$ m).

### Sensitivity testing

Sensitivity testing was done by well assay technique (Chung *et al.*, 1990). One ml of an overnight culture in TSB was inoculated into 100 ml of a TSA maintained at 45°C. Approximately 25 ml was poured into a petri dish and allowed to solidify. Wells (4 mm in diameter) were cut into the agar and filled with 40  $\mu$ l of filtered aqueous extracted of spices and medicinal plants. All plates were incubated at 37°C for 48 hr and the diameters of the inhibition zones were measured.

### Minimum inhibitory concentrations (MICs)

MICs were determined according to the method of Richards *et al.* (1995). Briefly, 9.9 ml volumes of TSB containing a series of dilutions of cloves, cinnamon, roselle, leadwort, pomegranate, sappan and betle leaf were inoculated with 0.1 ml from an 18 hr culture to give approximately  $3.0 \times 10^4$  or  $3.0 \times 10^6$  cfu/ml *E. coli* O157 : H7 and  $6.0 \times 10^4$  or  $6.0 \times 10^6$  cfu/ml *Y. enterocolitica*. The inoculated tubes were incubated for 24 hr at 37°C and the lowest concentrations showing no growth in tubes were recorded as the MICs.

### Effects of pH and temperature on the antibacterial activity of plant extracts

(i) pH. TSB (100 – ml portions) was adjusted to pH 4.5, 5.5, 6.5, 7.5, 8.5 and 9.5 with 1 N hydrochloric acid or 1 N sodium hydroxide and sterilized for 15 min 121°C. Filtrate of plant extracts were added to obtain the concentration of 500  $\mu$ g/ml. The suspensions of bacteria tested were added to provide a population of  $10^4$  cfu/ml in each flask, then incubated at 37°C for 48 hr. Samples were taken and monitored by spread plating on TSA. The number of cfu/ml was determined after plates were incubated for 48 hr at 37°C.

(ii) Temperature. TSB (100 – ml portions) pH 7.3 were used in the experiment. Filtered plant extracts and cultures of tested bacteria were added,

incubated at 4, 10, 25, 35 and 45°C for 48 hr, then assayed as described above for the pH study.

## RESULTS AND DISCUSSION

Of the 22 spices and medicinal plants extracts tested, cloves showed high inhibitory effect against those two organisms, *E. coli* O157 : H 7 and *Y. enterocolitica* (Table 1). It could produce clear zones up to  $17.75 \pm 0.09$  mm. and  $18.00 \pm 0.58$  mm. against *E. coli* O157 : H 7 and *Y. enterocolitica*, respectively. The essential oil, eugenol in cloves, has been reported to possess antibacterial activities

(Karapinar and Aktug, 1987). Cinnamon also showed high antibacterial activity against both organisms. Beuchat and Golden (1989) also reported that cinnamic aldehyde in cinnamon act as antibacterial.

The betle leaf showed the highest activity on *E. coli* O157 : H 7, where as the pomegranate rind exhibited the highest antibacterial action on *Y. enterocolitica*. Atal *et al.* (1975) reported that betle leaf contain many essential oils such as chavicol, allyl pyrocatechol, chavibetol, methyl chavicol, carvacrol, estragol, eugenol methyl ether and cadinene. Tuppenyuthpijarn *et al.* (1982)

**Table 1** Spices and medicinal plants used in the experiment.

Common name	Scientific name	Part extracted
Betle leaf	<i>Piper betle</i> L.	Leave
Black pepper	<i>Piper nigrum</i> Linn.	Seed
Cardamon	<i>Amomum krervanh</i> Pierre.	Fruit
Cinnamon	<i>Cinnamomum zeylanicum</i> Nees.	Stem
Cloves	<i>Eugenia caryophyllata</i> Thunb.	Flower
Creat	<i>Andrographis paniculata</i> Nees.	Leave
Cumin	<i>Cuminum cyminum</i> L.	Seed
Galanga	<i>Alpinia officinarum</i> Hance.	Underground stem
Galingale	<i>Boesenbergia pandurata</i> Holtt.	Root
Ginger	<i>Zingiber officinale</i> Rosc.	Underground stem
Jackfruit heartwood	<i>Artocarpus heterophyllus</i> Lamk.	Stem
Leadwort	<i>Plumbago indica</i> Linn.	Root
Neem tree	<i>Azadirachta indica</i> A.	Leave
Nutmeg	<i>Myristica fragrans</i> Linn.	Fruit
Pomegranate	<i>Punica granatum</i> Linn.	Fruit rind
Roselle	<i>Hibiscus sabdariffa</i> Linn.	Fruit
Safflower	<i>Carthamus tinctorius</i> Linn.	Flower
Sappan	<i>Cassalpinia sappan</i> Linn.	Stem
Star anise	<i>Illicium verum</i> Hooker.	Flower
Tea	<i>Camellia sinensis</i> Ktze.	Leave
Tumeric	<i>Curcuma longa</i> Linn.	Underground stem
White pepper	<i>Piper nigrum</i> Linn.	Seed

revealed that the essential oils from betle leaf have been shown of having antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

The pomegranate tree is a tannin bearing plant. Its fruit rind has been shown to be rich in tannins as well (Kitanov *et al.*, 1980). Tannins have been reported to be bacteriostatic or bactericidal for *S. aureus*, *Streptococcus pneumoniae*, *Bacillus antracis*, *Listeria*

*monocytogenes* and *Pseudomonas fluorescens* (Beuchat and Heaton, 1975; Chung *et al.*, 1993).

MIC values of crude plant extracts toward *E. coli* O157 : H 7 and *Y. enterocolitica* were determined and are presented in Tables 3 and 4. These results were consistent with the well assay method. Betle leaf showed maximal inhibitory effect against *E. coli* O157 : H 7 with MIC of 312 and 625 µg/ml according to the inoculum sizes of

**Table 2** Biological activity of various spices and medicinal plants against *E. coli* O157 :H 7 and *Y. enterocolitica*.

Spices and medicinal plants	Diameter of inhibition zone (mm) ± S.D. <sup>a</sup>	
	<i>E. coli</i> O157 : H 7	<i>Y. enterocolitica</i>
Betle leaf	17.90 ± 0.45	- <sup>b</sup>
Black pepper	-	-
Cardamon	-	-
Cinnamon	11.25 ± 0.43	15.00 ± 0.87
Cloves	17.75 ± 0.09	18.00 ± 0.58
Creat	-	-
Cumin	-	-
Galanga	-	-
Galingale	-	-
Ginger	-	-
Jackfruit heartwood	-	-
Leadwort	-	10.25 ± 0.83
Neem tree	-	-
Nutmeg	-	-
Pomegranate	-	18.25 ± 0.50
Roselle	-	12.25 ± 0.18
Safflower	-	-
Sappan	-	15.00 ± 0.35
Star anise	-	-
Tea	-	-
Tumeric	-	-
White pepper	-	-

<sup>a</sup> Mean value of four determinations, each from a different plate

<sup>b</sup> No inhibition was detected ; the control diameter is 4 mm.

$3.0 \times 10^4$  and  $3.0 \times 10^6$  cfu/ml, respectively. Whereas pomegranate rind exhibited activity against *Y. enterocolitica* with MIC of 156 and 312  $\mu\text{g}/\text{ml}$  according to the inoculum sizes of  $6.0 \times 10^4$  and  $6.0 \times 10^6$  cfu/ml, respectively.

The influences of pH on the antibacterial activity of betle leaf on the growth of *E. coli* O157 : H 7 and pomegranate rind on the growth of

*Y. enterocolitica* are shown in Figure 1. The results revealed that the inhibitory effect is much greater for plant extracts at lower pH than the effect of the same concentration of plant extracts at higher pH against test organisms. It should be noted that *E. coli* O157 : H7 and *Y. enterocolitica* are able to grow over a pH range from approximately pH 4.5 to 9.5 (Doyle *et al.*, 1997; Robins – Browne, 1997).

**Table 3** MIC determinations for spices and medicinal plants in TSB against *E. coli* O157 : H 7.

Spices and medicinal plants	<i>E. coli</i> O157 : H 7 inoculum size (cfu / ml)	MIC ( $\mu\text{g} / \text{ml}$ )
Betle leaf	$3.0 \times 10^4$	312
	$3.0 \times 10^6$	625
Cinnamon	$3.0 \times 10^4$	5000
	$3.0 \times 10^6$	5000
Cloves	$3.0 \times 10^4$	1250
	$3.0 \times 10^6$	2500

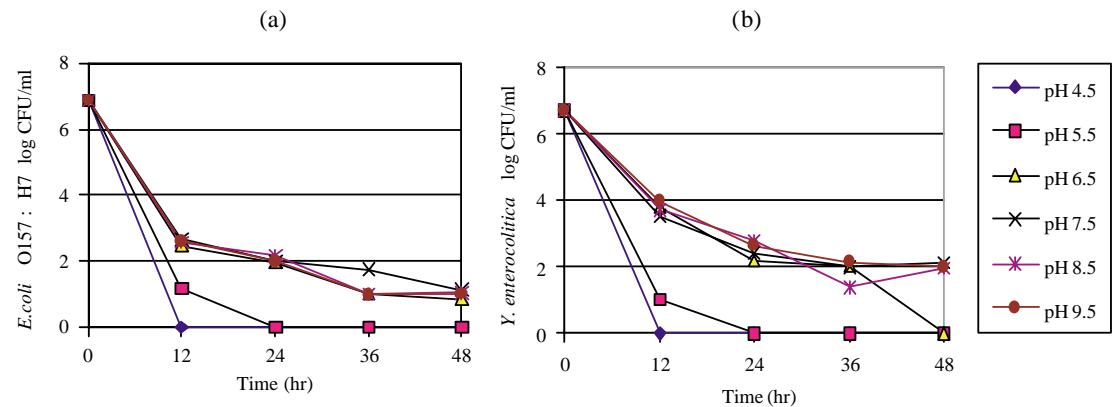
**Table 4** MIC determinations for spices and medicinal plants in TSB against *Y. enterocolitica*.

Spices and medicinal plants	<i>Y. enterocolitica</i> inoculum size (cfu / ml)	MIC ( $\mu\text{g} / \text{ml}$ )
Cinnamon	$6.0 \times 10^4$	2500
	$6.0 \times 10^6$	2500
Cloves	$6.0 \times 10^4$	625
	$6.0 \times 10^6$	1250
Leadwort	$6.0 \times 10^4$	1250
	$6.0 \times 10^6$	2500
Pomegranate rind	$6.0 \times 10^4$	156
	$6.0 \times 10^6$	312
Roselle	$6.0 \times 10^4$	>5000
	$6.0 \times 10^6$	>5000
Sappan	$6.0 \times 10^4$	625
	$6.0 \times 10^6$	1250

This indicates that the inhibitory effect is a plant extracts phenomenon and is not a result of the pH of the medium.

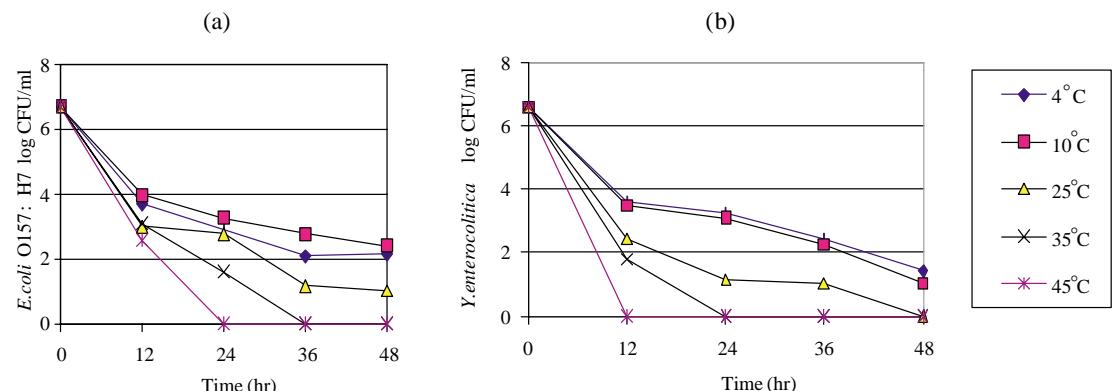
From Figure 2 it can be seen that plant extracts were less inhibitory against *E. coli* O157 : H7 and *Y. enterocolitica* at 4 and 10°C with an increase in the bactericidal activity compared to the

same concentration at 25, 35 and 45°C. Doyle *et al.* (1997) and Robins – Browne (1997) also reported that *E. coli* O157 : H7 and *Y. enterocolitica* are able to replicate at 44°C and can survive up to 50°C. This also indicates that the antibacterial activity is a result of plant extracts, and is not a temperature affects on viability of microorganisms tested. Smith



**Figure 1** The effect of pH on survival of *E. coli* O157 : H7 and *Y. enterocolitica* in the presence of plant extracts.

- (a) *E. coli* O157 : H7, beetle leaf conc. 500 µg/ml
- (b) *Y. enterocolitica*, pomegranate rind conc. 500 µg/ml



**Figure 2** The effect of temperature on survival of *E. coli* O157 : H7 and *Y. enterocolitica* in the presence of plant extracts.

- (a) *E. coli* O157 : H7, beetle leaf conc. 500 µg/ml
- (b) *Y. enterocolitica*, pomegranate rind conc. 500 µg/ml

- Palmer *et al.* (1998) also reported that at 35°C *L. monocytogenes* was extremely sensitive to the oil of nutmeg when compared to 4°C.

The above findings advocate the further investigation of both betle leaf and pomegranate rind extracts. It will be necessary to identify the active compounds or components and to evaluate their potential for use as antimicrobial agents.

## CONCLUSION

The betle leaf (*Piper betle*) had the highest antibacterial activity against *E. coli* O157 : H 7, whereas the pomegranate rind (*Punica granatum* Linn.) exhibited highest antibacterial action on *Y. enterocolitica*. Its antibacterial action depends mainly on pH and temperature of the medium. The antibacterial of plant extracts decrease as the pH of medium increased. At low temperatures (4 and 10°C) the plant extracts were less effective than at high temperatures (25, 35 and 45°C).

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