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Antimicrobial effect of Malaysian vegetables against enteric bacteria

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ABSTRACT

Objective: To investigate the antibacterial activities of green vegetables (pennywort, mint, garlic, parsley and celery) against four common enteric bacteria [*Salmonella enterica* (ATCC 25957) (*S. enterica*), *Shigella flexneri* (ATCC 12022) (*S. flexneri*), *Escherichia coli* (ATCC 43889) (*E. coli*) and *Enterobacter cloacae* (ATCC 13047) (*E. cloacae*)] as an alternative medicine for controlling food borne diarrhea disease and the synergistic effect of green vegetables against those bacteria.

Methods: Five common vegetables (pennywort, mint, garlic, parsley and celery) were purchased and extracted. The antimicrobial activities of these extracts were tested against four common enteric bacteria (*S. enterica*, *S. flexneri*, *E. coli* and *E. cloacae*). Ten different concentrations of the extracts (from 640 to 1.25 mg/mL) were prepared and used for the study. The minimal inhibitory concentration (MIC) was determined by the broth dilution method. The antimicrobial activities were assessed by using both well diffusion and disc diffusion methods.

Results: Garlic extract showed excellent inhibitory effects on all enteric bacteria. Other plants (parsley, celery, mint and pennywort) were not effective against enteric bacteria. The MIC of garlic against *S. flexneri* and *E. cloacae* was 40 mg/mL. The MIC of *S. enterica* and *E. coli* were 20 and 10 mg/mL, respectively. The performance of the well diffusion method was better than that of the disc diffusion method with clear and sharp inhibition zones of tested bacteria against plant extracts.

Conclusions: Garlic had excellent antimicrobial effects against enteric bacteria and was recommended to be given to patients with gastroenteritis. The other vegetables (pennywort, mint, parsley and celery) showed no inhibitory effects on enteric bacteria but still can be used for its richness in vitamins and fibers. The performance of the well diffusion method was better than that of the disc diffusion method in detecting the antibacterial effects of green vegetables.

1. Introduction

Herbal plants are used globally due to its antimicrobial effects and become very important due to the increasing percentage of drug-resistant pathogens [1]. However, antibiotics abuse has become the major factor for the emergence and dissemination of multidrug resistant strains of several groups of microorganisms [2]. Antimicrobial effects of different plants and their derivatives have been studied earlier [3]. Plant oils and extracts have been used for many thousands of years in food preservation, pharmaceuticals, alternative medicine and natural therapies [4]. Even different vegetables have

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antimicrobial, anti-inflammatory and antioxidant properties [5]. Previous studies have shown the antibacterial activities of different plants against different enteric bacteria by using plant extracts [6]. Celery (*Apium graveolens*) is used commonly as vegetable in cooking in various countries including Malaysia. Celery oil had potent antimicrobial activity against various pathogenic and saprophytic microorganisms including *Bacillus subtilis*, *Eschericia coli* (*E. coli*) and *Saccharomyces cerevisiae* [7]. Garlic (*Allium sativum*) is a perennial bulb forming plant which belongs to the genus *Allium* in the family Liliaceae. The garlic is therapeutically effective because of its oil and water soluble organosulfur compounds [8]. Thiosulfinates (allicin) acts by inhibiting both RNA and DNA synthesis then inhibiting the protein synthesis [9]. Mint leaves (*Mentha asiatica*) have been traditionally used in folk medicine and believed to have antimicrobial activities. Menthol is the active ingredient of the mint leaves and doubts about its disinfecting and antimicrobial effects. Studies confirmed the antimicrobial activities of the mint against *Staphylococcus aureus* and *E. coli* [10]. Pennywort (*Centella asiatica*) is a small herbaceous annual plant with small-sized leaves and short petiole stem. It grows in damp swampy areas in tropical and sub-tropical regions including Malaysia [11]. It has been used for centuries as a traditional medicine in India and oriental countries for treatment of mental, fatigue, anxiety, epidermal wound, eczema and leprosy. And the most prominent group of biologically active compounds is the triterpenes which consist of asiatic acid, madecassic acid and asiaticoside [12]. Asiatic acid is an aglycone of asiaticoside isolated from the plant *Centella asiatica*, commonly used for wound healing, antimicrobial, antioxidant and anti-free radical protection, dermis reconstruction by stimulating the collagens synthesis in addition to anti-aging effects by reinforcing the biomechanical properties of mature skins [13]. Other plant is parsley (*Petroselinum crispum*) which is culinary herb commonly used to flavor the cuisines of Southeast Asian countries. Moreover, parsley is a rich source of certain vitamins and minerals and widely used by diabetic patients to reduce blood glucose [14]. Parsley showed antimicrobial effect against some Gram-negative bacteria and had anti-adhesive effects against *Helicobacter pylori* [15]. It has also been found useful as a diuretic, laxative and possesses antioxidant activity [14]. Clearly, there were few studies which targeted the antimicrobial effects of some vegetables (pennywort, mint, garlic, parsley and celery) against certain enteric bacteria. Therefore, this study aimed to determine the antimicrobial effects of some vegetables used in Malaysian food against selected enteric bacteria (*Salmonella*, *Shigella*, *E. coli* and *Enterobacter*), which cause food poisoning and gastroenteritis.

2. Materials and methods

2.1. Bacterial strains

Four standard bacterial strains which caused diarrhea among populations were used as tested microorganisms. All microorganisms were obtained from Microbiology Laboratory at Institute of Medical Molecular Biotechnology, Faculty of Medicine, AMARA University of Technology. Bacterial strains used in this study were *Salmonella enterica* (ATCC 25957) (*S. enterica*), *Shigella flexneri* (ATCC 12022) (*S. flexneri*), *E. coli* (ATCC 43889) and *Enterobacter cloacae* (ATCC 13047) (*E. cloacae*). All bacterial strains have been inoculated in blood agar and incubated for 24 h at 37 °C. The bacterial suspension was prepared by inoculating two bacterial colonies in trypticase soy broth for 3 h at 37 °C and the turbidity was adjusted in phosphate buffered saline to 0.5 McFarland's scale.

2.2. Plant collection and extraction of green vegetables

The study plants (pennywort, mint, garlic, parsley and celery) were purchased from a nearby market. The plants were washed with tap water, followed by detergent, salt, ethanol and distilled water then the plants were dried in an incubator. About 640 g of each plant were blended together with 100 mL sterile distilled water to form a mixture with a concentration of 640 mg/mL. Two times filtration of each extract was done to obtain the clear and pure extract which later was kept in the refrigerator at 4 °C. Then, 1 mL of 640 mg/mL of each extract was mixed with 9 mL sterile distilled water in falcon tube and stirred by using vortex mixture. Later on, a two-fold serial dilution was done for each extract to achieve an extract concentration ranging from 640 to 1.25 mg/mL.

2.3. Antimicrobial susceptibility testing

2.3.1. Agar well diffusion method

Agar well diffusion method was used to determine the antimicrobial activity of green vegetables. One hundred microliters of bacterial suspension was spread on Muller-Hinton agar (MHA) plates containing 6 mm wells. Fifteen microliters of each extract was poured into each well and plates were incubated at 37 °C aerobically for 24 h. The diameter of the growth inhibition zone around the wells was measured in millimeter and recorded. Wells containing plant extract with no inhibition zones were considered as negative results. Both ampicillin (10 μg) and chloramphenicol (30 μg) were used as a control.

2.3.2. Disc diffusion method

Ready discs were labeled for each plant extract concentration accordingly. Then, the discs were autoclaved and each disc was infused with 15 μL of each extract concentration. One hundred microliters of each bacterial suspension was inoculated onto the MHA and spread all over the agar surface by using sterile swab. Then, ten different concentrations of the extract discs together with the controls were transferred on the surface of MHA and incubated for 24 h within 37 °C.

2.4. Minimal inhibitory concentration (MIC)

Broth dilution assay was used to determine the MIC of the green vegetables against standard enteric bacterial strains as recommended by the Clinical Laboratory Standards Institute [16]. The concentrations of the extracts tested ranged from 640 to 1.25 mg/mL. This test was performed in sterile bijou bottles which were loaded with 100 μL of each extracted dilution into each bottle. Bacterial inoculums (100 μL) containing 5×10^5 CFU of each microorganism were added to each bottle. In each panel of the tested extract, a positive control

(without extract) and negative control (no inoculum) were added. All bottles were aerobically incubated at 37 °C. After incubation for 24 h, the bacterial growth was assayed by its visible turbidity. The highest dilution of the extract which showed no visible bacterial growth and no turbidity in bijou bottle was considered as MIC. After 24 h of incubation, 100 µL of each mixture was pipetted and inoculated on blood agar and spread uniformly with the sterile spreader and again incubated for 24 h at 37 °C. On the next day, all blood agars were examined and all bacterial colonies were counted and recorded.

3. Results

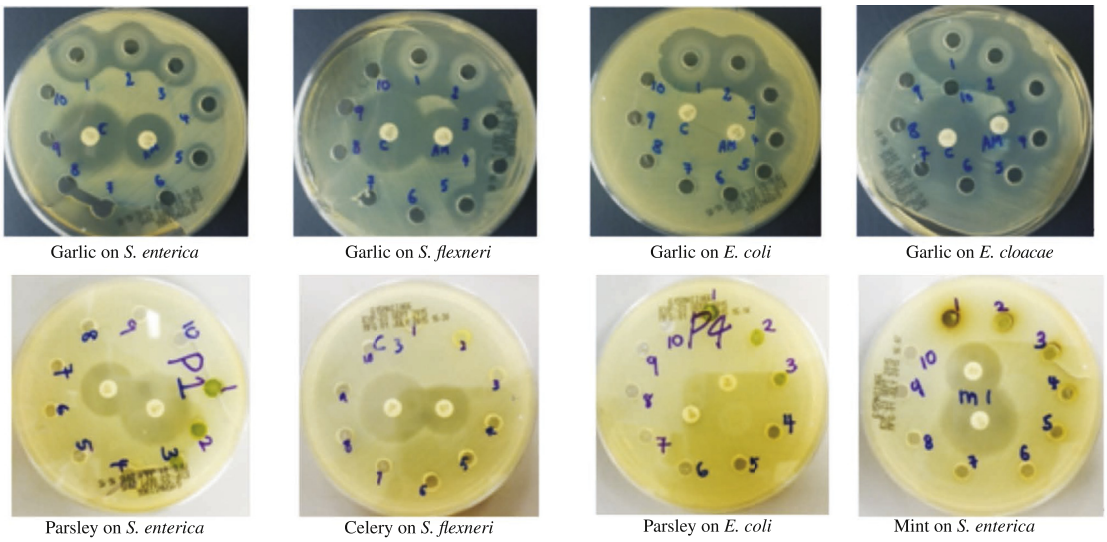
The antimicrobial effects of green vegetables against different enteric bacteria were summarized in Table 1. Garlic extract showed excellent inhibitory effects on all enteric bacteria at various concentrations (40–640 mg/mL). However, the other plants (parsley, celery, mint and pennywort) did not show inhibitory effects as seen in Figure 1. The MIC of garlic against *S. flexneri* and *E. cloacae* was 40 mg/mL (Figure 2) while those for *S. enterica* and *E. coli* were 20 and 10 mg/mL, respectively (Table 2). However, the result couldn't show MIC for parsley, celery, mint and pennywort against enteric bacteria. Chloramphenicol disc was used as a positive control and showed inhibition in the growth of *S. enterica*, *S. flexneri* and *E. cloacae* while ampicillin disc was effective against *S. enterica* and *S. flexneri* only. Our results also showed that the performance of the agar well diffusion method was better than that of the disc diffusion method with clear and sharp inhibition zones of tested bacteria against plant extracts (Figure 3).

Table 1

Antibacterial effects of green vegetables and standard antibiotics by agar well diffusion method.

Vegetables	Tested organisms	Diameter of inhibition zone (mm)											
		640	320	160	80	40	20	10	5	2.5	1.25	AMP (10)	CHL (30)
Parsley	*S. enterica*	0	0	0	0	0	0	0	0	0	0	20	35
	S. flexneri	0	0	0	0	0	0	0	0	0	0	20	27
	E. coli	0	0	0	0	0	0	0	0	0	0	0	0
	E. cloacae	0	0	0	0	0	0	0	0	0	0	0	23
Celery	*S. enterica*	0	0	0	0	0	0	0	0	0	0	20	25
	S. flexneri	0	0	0	0	0	0	0	0	0	0	18	25
	E. coli	0	0	0	0	0	0	0	0	0	0	0	0
	E. cloacae	0	0	0	0	0	0	0	0	0	0	0	27
Garlic	*S. enterica*	20	17	15	12	10	0	0	0	0	0	18	24
	S. flexneri	26	22	20	17	12	0	0	0	0	0	19	27
	E. coli	23	20	15	13	9	0	0	0	0	0	0	0
	E. cloacae	30	26	24	18	14	0	0	0	0	0	9	25
Pennywort	*S. enterica*	0	0	0	0	0	0	0	0	0	0	20	30
	S. flexneri	0	0	0	0	0	0	0	0	0	0	30	20
	E. coli	0	0	0	0	0	0	0	0	0	0	0	0
	E. cloacae	0	0	0	0	0	0	0	0	0	0	0	27
Mint	*S. enterica*	0	0	0	0	0	0	0	0	0	0	20	22
	S. flexneri	0	0	0	0	0	0	0	0	0	0	18	27
	E. coli	0	0	0	0	0	0	0	0	0	0	0	0
	E. cloacae	0	0	0	0	0	0	0	0	0	0	0	26

The concentration of different extract and antibiotic discs was expressed as mg/mL. AMP: Ampicillin; CHL: Chloramphenicol.



Garlic on *S. enterica* | Garlic on *S. flexneri* | Garlic on *E. coli* | Garlic on *E. cloacae*

Parsley on *S. enterica* | Celery on *S. flexneri* | Parsley on *E. coli* | Mint on *S. enterica*

Figure 1. Antimicrobial effects of green vegetables on enteric bacteria.

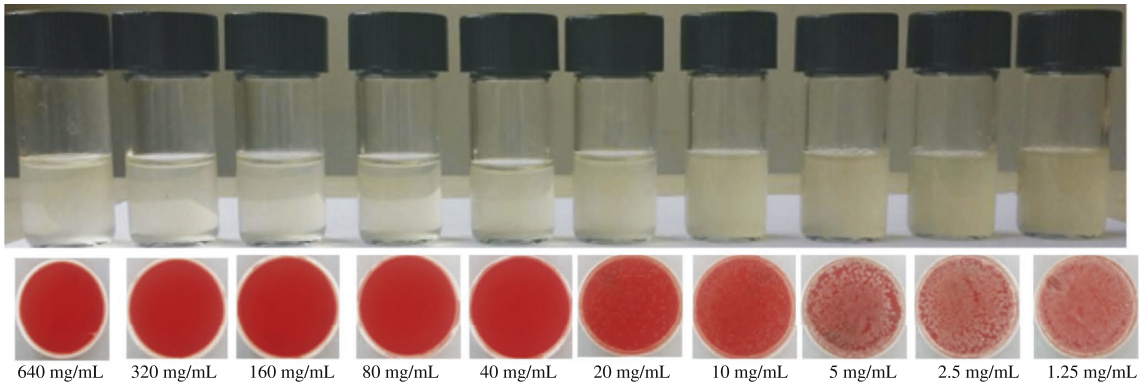
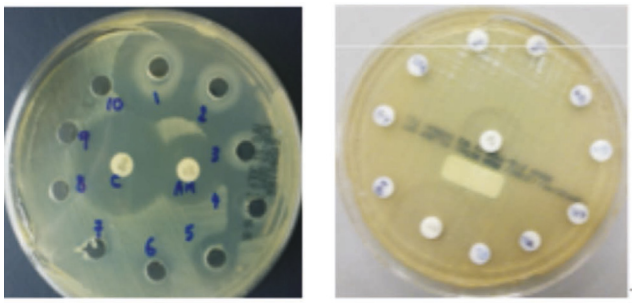


Figure 2. MIC of garlic against *S. flexneri* by the broth dilution method and blood agar.

Table 2
The MIC of green vegetables against enteric bacteria.

Vegetables	*S. enterica*	*S. flexneri*	*E. coli*	*E. cloacae*
Parsley	++	++	++	++
Celery	++	++	++	++
Garlic	20 mg/mL	40 mg/mL	10 mg/mL	40 mg/mL
Pennywort	++	++	++	++
Mint	++	++	++	++

++: Full growth of bacteria seen on bijou bottles and blood agar plates.



Garlic on *S. flexneri* by well method Garlic on *S. flexneri* by disc method

Figure 3. Performance of well and disc diffusion methods for detection of the antibacterial activity of garlic against *S. flexneri*.

4. Discussion

Antibiotic resistance of enteric bacteria to common antibiotics is the main cause of treatment failure [17]. Medicinal herbs are used in treating different infections. In this study, five plants were tested against enteric bacteria causing gastroenteritis. The results of antibacterial susceptibility testing showed that garlic had a potent antimicrobial effect against *S. enterica*, *S. flexneri*, *E. coli* and *E. cloacae* at different concentrations. Previous studies revealed that it was effective against a wide variety of microbial pathogens [18]. In 2012, Gull *et al*. observed a significant bactericidal effect of garlic extracts against *Staphylococcus epidermidis* and *Salmonella typhi*. Even bacteria which showed resistance to different antibiotics were sensitive to garlic extracts [19]. Numerous studies have proven the antimicrobial activities of plant extracts against food borne pathogens [20]. However, the results achieved are difficult to compare directly, usually because of the low number of plant samples tested, different test methods, and diverse bacterial strains and sources of antimicrobial samples used [21]. The observed resistance of some bacteria such as *Salmonella*, *Shigella*, *Escherichia* and *Enterobacter* could be due to low concentrations of extracts used or high concentration of the bacteria suspension used. In this study, we used fresh plant in experiment which could reflect the actual antibacterial effect of different extracts in comparison with previous studies which used the dry powder of plants [22]. Garlic has high antibacterial properties on a wide spectrum of enteric bacteria which could be due to the chloroform extract or the essential oil of garlic which had more antibacterial properties than garlic powder against enteric bacteria [23]. Our study revealed the effectiveness of the antibacterial properties of garlic juice on enteric bacteria. Similar to our study results, Saravanan *et al*. in India concluded that the aqueous extract of garlic had a great effect on the target bacteria as the growth of bacteria was inhibited within 14 h [24]. However, other plants (parsley, celery, mint and pennywort) did not show significant inhibitory effects on enteric bacteria which might be due to low concentration of extracts used or high concentration of bacteria suspension used. Based on the broth dilution assays, the MIC of 40 mg/mL could be used as an antibacterial agent against four major enteric bacteria responsible for gastroenteritis. Our study confirms the better performance of agar well diffusion method in the detection of the antimicrobial effects of green vegetables in comparison with disc diffusion method. In this experiment, the same concentration of extract and bacterial suspension was used to avoid bias. The poor performance of disc method could be attributed to low diffusion of the extract on the agar surface. Further studies using standard antibiotics with known concentrations might confirm the performance of each method. The current study concludes and confirms that garlic has excellent antimicrobial effects against enteric bacteria and is recommended to be given to patients with gastroenteritis either with food or in capsule form. Pennywort, mint, parsley and celery showed no inhibitory effect on enteric bacteria but still can be used for its richness in vitamins and fibers. The performance of agar well diffusion method was better than that of disc diffusion method in the detection of the antibacterial effects of green vegetables.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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