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# Antimicrobial potential of essential oils from herbs and spices used in traditional Malaysian cuisine against selected food-borne pathogens

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## Abstract

The essential oils from spices and herbs are the most promising natural antimicrobials, because they do not cause microbial resistance due to the diversity of mechanisms of action. In order to reduce case of food-borne pathogen contamination using a natural compound, the antimicrobial activity of herbs and spices used in Malaysian cuisine were tested against *Salmonella enterica*. Twenty five types of essential oils were successfully extracted by hydro-distillation method and the inhibition performance on *S. enterica* was evaluated in vitro by disc diffusion method and the minimum inhibitory concentrations were determined. Result showed that cinnamon, cloves and turmeric leaves essential oils were potentially effective against *S. enterica* at inhibition percentage of above 50%. The minimum inhibition concentrations ranged from 3.13 x $10^{-2}$ to 1.73 x $10^{-4}$ mg/mL. Cinnamon essential oils were also found to be effective against *Escherichia coli* and *Staphylococcus aureus*. These oils proved to be potentially effective as natural alternative to prevent food poisoning or as food preservatives.

Keywords: food-borne pathogen, essential oils, herbs, spices, antimicrobial

## Introduction

Food-borne illness like cholera, dysentery, typhoid fever, hepatitis A and food poisoning, are a growing public health problem worldwide that causes a significant impact on the economy and trade in Malaysia and other developing countries (Meftahuddin 2002; Soon et al. 2011; Sharifa Ezat et al. 2013). Ingestion of food contaminated with microorganisms and chemicals resulted in this food-borne illness, which presented a common clinical presentation in the form of gastrointestinal symptoms. Worse, these might also cause multi-organ failure and even cancer, resulting in a substantial burden of disability and mortality (Sharifa Ezat et al. 2013). Several pathogenic microorganisms, such as *Escherichia coli*, *Listeria monocytogenes, Staphylococcus aureus*, *Salmonella enterica, Klebsiella pneumoniae*, *Salmonella typimurium* and *Campylobacter jejuni* have been reported as causal agents of food-borne diseases or food spoilage, which is a significant concern in the food industry (Deak and Beuchat 1996; Lim 2002; Soon et al. 2011). In Malaysia, cases of food-borne illness are not rare due to the condition are suitable in facilitating the growth of most bacteria. It is reported in 2010, the food and water-borne disease were low, ranging from 1.56 to 0.14 cases per 100,000 populations. However, food poisoning incidents have been on the rise with rates of 36.17 and 44.18 per 100,000 populations in 2009 and 2010, respectively (Sharifa Ezat et al. 2013).

Since the prehistoric times, herbs and spices have been utilised to enhance the flavor and taste of cooked food. Usually, spices are used in small quantities as seasoning agent due to its pungent aroma. Whereas herbs are condiments that are used in larger quantities than spices to compliment the foods. Although the scientific reasons were unknown, herbs and spice has been recognised for its medicinal value since ancient times especially in countries like India, China and South-East Asia, where they play significant roles in cultural heritage (D'souza et al. 2017). A review by Ramsey et al. (2020) concluded that a few herbs and spices possess antimicrobial activity against selected microorganism such as thyme, oregano, garlic, lemon balm and cinnamon. Other than the antimicrobial activity, many spices and herbs such as basil, clove, citrus, garlic, cinnamon, fennel, cumin lemongrass, oregano, rosemary and thyme possessed significant antifungal

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activities against selected pathogenic fungal (Liu et al. 2017; Ramsey et al. 2020). The antibacterial activity may vary among different strains of the same species and the form used (eg., dried, fresh or extracted), as well as harvesting seasons and geographical area (Nanasombat and Lohasupthawee 2005).

Malaysian cuisine is a unique blend of traditional dishes influenced by cooking culture from India, China, the Middle East, Indonesia and Europe. The country's diverse geography and history played a significant role in shaping its cuisine with various herbs and spices contributing to the flavor and complexity of Malaysian dishes. Some of the most commonly used herbs and spices include turmeric, galangal, lemongrass, chilies, coriander, cinnamon, cloves, cumin, star anise, fennel and many more. These herbs and spices are often used in combination with each other to create the unique flavors associated with Malaysian cuisine. Examples of dishes with these herbs and spices include curries, stews, soup and stir-fried dishes.

Essential oil are powerful natural plant products that have been recognised since ancient years for their medicinal value. They are basically natural oils obtained from aromatic plants and herbs through various methods. In ancient Egypt, oils were primarily extracted through the infusion method (Djilani and Dicko 2012). However, with the influence of the Greeks and Romans, the introduction of distillation techniques added further significance to essential oils, thus enhancing their value and utilization. These techniques were further refined with the arrival of Islamic civilisation (Djilani and Dicko 2012). As the scientific advancements progressed during the Renaissance era, there was comprehensive exploration and understanding of the composition and characteristics of essential oils by Europeans (Djilani and Dicko 2012). According to Aziz et al. (2018), in the current world of essential oils extraction, they can be categorised into two method which are classical (eg., hydrodistillation, steam distillation, hydrodiffusion, and solvent extraction) and innovative (eg., supercritical fluid extraction, subcritical extraction liquid, solvent-free microwave extraction).

Essential oils, known as plant secondary metabolites, are a complex mixture of volatile organic compounds that include terpenes and sesquiterpenes (hydrocarbon group), as well as alcohols (geraniol, a-bisabolol), ethers, esters (g-tepinyl acetate, cedryl acetate), ketones (menthone, *p*-vetivone), aldehydes (citronellal, sinensal), phenols (thymol), lactones and phenol ester (oxygenated group) (Dhifi et al. 2016; Aziz et al. 2017). Generally, there are two or three major components present in essential oils, and these components play a crucial role in determining the biological properties of these aromatic oils. There are more than 200 constituents found in various essential oils, and among the main specific groups are terpenoids, phenylpropanoids and short-chain aliphatic hydrocarbon derivatives (Aziz et al. 2017). The chemical properties of essential oils are affected by the extraction process method employed, which influenced not only the number and type of molecules, but also their stereochemical structures (Aziz et al. 2017). Various other factors can alter the quality, quantity and composition of extracted essential oils, including the plan organ, degree of maturity, soil composition, genetics, age, geographic and climate (Dhifi et al. 2016; Aziz et al. 2017).

Various studies have been reported on the benefits of essential oils application as natural alternatives to synthetic antimicrobials against viruses, bacteria, fungi and yeast (Muthuswami et al. 2008; Tzortzakis 2009; Usha et al. 2012; Vazirian et al. 2015; D' Souza et al. 2017; Aziz et al. 2018; Ramsey et al. 2020). The application of essential oils not only as antimicrobial agent, but also possess functional properties for health such as anticancer, anti-aging, cardiovascular diseases, antioxidant and anti-inflammatory (Djilani and Dicko 2012; Tanu and Harpreet 2016; Aziz et al. 2018).

The aim of this research was to determine the antimicrobial activity of the essential oils from identified herbs and spices that are commonly used in Malaysian cuisine against selected food-borne pathogen, *Salmonella enterica*.

## Materials and methods

### *Plant materials*

Several types of herbs and spices were subjected to this study. *Elettaria cardamomum* (cardamom), *Syzygium aromaticum* (clove) and *Cinnamomum verum* (cinnamon), *Coriander sativum* (coriander seed and leaves), *Citrus hystrix* (kaffir leaves and fruits), *Cuminum cyminum* (cumin), *Curcuma longa* (turmeric leaves and rhizome), *Cymbopogon citratus* (lemongrass), *Illicium verum* (star anise), *Allium sativum* (garlic), *Myristica fragrans* (nutmeg), *Etlingera elatior* (torch ginger), *Syzygium polyanthum* (bay leaves), *Ocimum basilicum* (basil leaves), *Zingiber officinale* (ginger), *Foeniculum vulgare* (fennel), *Piper nigrum* (blackpepper), *Alpinia galanga* (galangal), *Citrofortunella microcarpa* (calamansi), *Mentha arvensis* (mint), *Rosmarinus officinalis* (rosemary) and *Etlingera coccinea* (tuhau) were purchased from local markets and grocery stores.

### *Preparation of extracts*

One hundred grams of each sample were subjected to hydro-distillation using a Clevenger-type apparatus. The samples were extracted with 900 mL of distilled water for 4 h or until no more essential oil was obtained. The essential oil was collected, and the water was separated from the oil and was stored separately at 4 °C in an amber bottle until further used.

### *Use of standardised bacterial colony numbers*

A standardised number of colony forming units (CFU) was obtained using McFarland standards. Five to ten colonies of a single morphological type from a 16 – 24 h agar plate were inoculated into a tube containing 5.0 mL of

Muller Hinton broth. The culture was incubated at 35 °C for 16 h with shaker incubator to promote uniformity and optimized growth. After 16 h of incubation, the turbidity of the broth culture was adjusted with sterile saline (0.9% w/v sodium chloride) to obtain a turbidity of 0.5 McFarland turbidity standards (1.5 x $10^8$ CFU/mL).

### *Preparation of bacterial culture*

The bacteria strains used in this study are known to be the cause of disease in human. *Salmonella enterica, Listeria innocua, Escherichia coli and Staphylococcus aureus* were used in the antibacterial assay. The bacteria were grown and maintained on Nutrient Agar (NA) and then stored at 4 °C under aerobic conditions. The bacteria were cultured overnight at 35 °C in nutrient broth, which was then adjusted to obtain turbidity was adjusted to match that of 0.5 McFarland standards ($10^6$ CFU/mL). Bacterial cultures were obtained from Biohazard Laboratory, Food Science and Technology Research Centre, MARDI.

### *Antimicrobial activity of essential oils*

The Kirby-Bauer disc diffusion susceptibility test was employed to determine the sensitivity of selected bacterial strains. The assay was based on the method described by Damjanovic-Vratnica et al. (2011) with slight modifications. Mueller-Hinton agar (20 mL) was poured into the petri plates and allowed to solidify. Two hundred microliters µL of inoculums were spread evenly over the surface of the media using a sterile cotton swab. Discs impregnated with 10 µL of essential oil samples were then applied firmly to the inoculated agar surface using sterile forceps and pressed gently to ensure contact with the surface. The plates were incubated for 24 h at 37 °C. For each petri dish, three discs were applied (test sample, positive control and negative control). The activity was measured as the diameter of clear zone surrounding the disc in milimeters (mm). The antimicrobial activity was expressed as the percentage of inhibition of the sample compared to the positive control (tetracycline). A blank disc served as negative control for this experiment. All tests were performed in triplicate.

### *Minimum inhibitory concentration (MIC)*

The minimum inhibitory concentration (MIC) was determined through the serial dilution method, using 96-well micro-plates according to the protocol by CLSI (2012) and Sarker et al. (2007) with slight modifications. All tests were performed in Mueller-Hinton Broth (MHB). A volume of 100 µL of the stock concentration of essential oil was added to the first row of the plate, while 50 µL of MHB was added to other wells. Serial dilutions were performed using the multichannel pipette and tips were discarded after use to ensure that each well had 50 µL of the test material in serially descending concentrations. To each well, 10 µL of resazurin indicator solution was added, followed by 30 µL of MHB using a pipette. Finally, 10 µL of bacterial suspension (5 x $10^6$ CFU/mL) was added to each well to achieve a concentration of 5 x $10^5$ CFU/mL. Each plate was loosely wrapped with cling film to prevent bacterial dehydration. Each plate included a set of controls: a column with a broad-spectrum antibiotic as positive control (tetracycline), a column with all solutions except the test compound, and a column with all solutions except the bacterial suspension (replaced with 10 µL of MHB instead). The plates were prepared in triplicate and placed in 37 °C incubator for 18 – 24 h. Colour changes were then assessed visually, and any color changes from purple to pink or colorless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value. The average of three values was calculated as the MIC for the test material and bacterial strain.

### *Statistically analysis*

The studies were performed in triplicate, and data were expressed as mean ± SEM (standard error of the mean).

## Results

### *Essential oil recovery*

The percentage of oil recovery presented in *Table 1* showing that the highest yields were obtained from nutmeg (4.45 – 12.31%), followed by cardamom (2.83 – 3.85%), clove (0.66 – 3.46%), cumin (0.94 – 2.40%), kaffir leaves (0.57 – 1.68%) and turmeric leaves (0.53 – 1.13%). According to Bowles (2003), essential oil production from plants is typically minimal, accounting for less than 1% of the total oil content. This finding supports the notion that most herbs and spices in this study have relatively low oil concentrations, rarely surpassing the 1% threshold. The data revealed different degree of oil recovery from each sample. In terms of physical properties, different oils exhibit different appearance, with some being oily, viscous, and fairly solid and others are watery. Most of the essential oils produced are colourless. Essential oils must be kept in amber bottle to preserve their delicate composition from the alteration by light.

### *Antimicrobial activity of selected essential oils against S. enterica*

As a preliminary step, the antimicrobial activities of 25 types of essential oils were determined by using paper disc diffusion method to screen the efficacy of essential oils among all samples. The antibacterial activity of essential oils against *S. enterica* was qualitatively and quantitatively assessed by evaluating the presence of inhibition zones and zone diameter measurements. Cinnamon oil exhibited the most promising antimicrobial activity by suppressing the growth of *S. enterica,* while cardamom and star anise were found to be the least effective.

Table 1. Herbs and spices selected for the essential oils extraction and the percentage of oil recovery

| **Family** | **Plant species** | **Common name** | **Local name** | **Part** | **Oil recovery (%)** |
|---|---|---|---|---|---|
| Apiaceae | *Coriandrum sativum* | Coriander seed | *Biji ketumbar* | Seeds | 0.17 – 0.37 |
| | | Coriander leaves | *Daun ketumbar* | Leaves | 0.03 – 0.05 |
| | *Cuminum cyminum* | Cumin | *Jintan putih* | Seeds | 0.94 – 2.40 |
| | *Foeniculum vulgare* | Fennel | *Jintan manis* | Seed | 0.92 – 2.42 |
| Zingiberaceae | *Elettaria cardomum* | Cardamom | *Buah pelaga* | Seeds | 2.83 – 3.85 |
| | *Curcuma longaa* | Turmeric leaves | *Daun kunyit* | Leaves | 0.53 – 1.13 |
| | | Turmeric | *Kunyit hidup* | Rhizome | 0.18 – 0.24 |
| | *Etlingera elatior* | Torch ginger | *Bunga kantan* | Stem | 0.03 – 0.08 |
| | *Zingiber officinale* | Ginger | *Halia* | Rhizome | 0.05 – 0.18 |
| | *Alpinia galanga* | Galangal | *Lengkuas* | Rhizome | 0.01 – 0.07 |
| | *Etlingera coccinea* | Tuhau | *Tuhau* | Stem | 0.05 – 0.10 |
| Myrtaceae | *Syzygium aromaticum* | Clove | *Cengkih* | Flower | 0.66 – 3.48 |
| | *Syzygium polyanthum* | Bay leaves | *Daun salam* | Leaves | 0.01 – 0.05 |
| Lauraceae | *Cinnamomum verum* | Cinnamon | *Kulit kayu manis* | Barks | 0.24 – 0.87 |
| Graminaceae | *Cymbopogon citrates* | Lemongrass | *Serai* | Stems | 0.31 – 0.64 |
| | *Citrus hystrix* | Kaffir leaves | *Daun limau purut* | Leaves | 0.57 – 1.68 |
| | | Kaffir lime | *Limau purut* | Fruit | 0.15 – 0.94 |
| Schisandraceae | *Illicium verum* | Star anise | *Bunga lawang* | Flowers | 0.24 – 0.58 |
| Amaryllidaceae | *Allium sativum* | Garlic | *Bawang putih* | Fruit | 0.07 – 0.31 |
| Myristicaceae | *Myristica fragrans* | Nutmeg | *Buah pala* | Seed | 4.45 – 12.31 |
| Lamiaceae | *Ocimum basilicum* | Basil leaves | *Daun selasih* | Leaves | 0.15 – 0.27 |
| | *Mentha arvensis* | Mint | *Daun pudina* | Leaves | 0.07 – 0.11 |
| | *Rosmarinus officinalis* | Rosemary | *Rosemari* | Leaves | 0.07 – 0.42 |
| Piperaceae | *Piper nigrum* | Black pepper | *Lada hitam* | Seed | 0.33 – 2.98 |
| Rutaceae | *Citrofortunella macrocarpa* | Calamansi | *Limau kasturi* | Fruit | 0.17 – 0.25 |

The effectiveness of the essential oils against *S. enterica* was summarised in *Table 2*. Among all the tested essential oils, cinnamon showed the best activity against *S. enterica* with an average inhibition percentage of 101.38%, followed by clove (62.5%), turmeric leaves (52.79%), cumin (45.83%), mint (45.83%) and lemongrass (42.37%). Other herbs and spices tested showed less than 10 mm inhibition zone with inhibition percentage below 40%. Based on the percentage of inhibition compared to the tetracycline, the activity was categorised as active or inactive, as shown in *Table 3*. Inhibitory percentages below 50% were considered as inactive. Therefore, only three essential oils were considered highly effective against *S. enterica.* Interestingly, the cinnamon oil was found to be similar to tetracycline in inhibiting the growth of *S. enterica.*

### *Minimum inhibitory concentration (MIC) of the effective essential oils*

The most effective essential oils with inhibition percentage above 50% from the disc diffusion method were then subjected to MIC assay using resazurin assay as described by Sarker et al. (2007). The resazurin assay is simple, sensitive, rapid, robust and reliable and could be successfully used to access antibacterial properties of natural products (Sarker et al. 2007). The effective concentrations of the essential oils were reported in *Table 4*. The minimum concentrations of essential oils required to inhibit the growth of *S. enterica* were 1.73 x $10^{-4}$ mg/mL for cinnamon, 4.88 x $10^{-4}$ mg/mL for clove and 3.13 x $10^{-2}$ mg/mL for turmeric leaves.

### *Antimicrobial activity of effective essential oils against other pathogen*

The three essential oils were then tested against other food-borne pathogen, namely *E. coli, S. aureus* and *L. innocua.* The data in *Table 5* shows that different inhibitory effects were exhibited by the essential oils against different pathogens, and even the effectiveness of tetracycline were different against *E. coli, S. aureus, L. innocua* and *S. enterica*, 27.33 ± 0.58 mm, 33.00 ± 1.00 mm, 38.58 ± 0.58 mm and 24.00 ± 0.52 mm, respectively. Cinnamon essential oil was not only effective against *S. enterica* but also highly effective against two other pathogens, *E. coli* and *S. aureus* with inhibition zones of 33.33 ± 0.58 mm and 32.67 ± 1.16 mm, respectively.

Table 2. Antimicrobial screening test of essential oils against *S. enterica*

| Sample | Inhibition zone (mm) | Inhibition (%) |
|---|---|---|
| Coriander seed | 9.00 ± 0.06 | 37.5 |
| Coriander leaves | 7.00 ± 1.00 | 29.17 |
| Cumin | 11.33 ± 0.15 | 45.83 |
| Fennel | 7.67 ± 0.58 | 31.96 |
| Cardamom | 7.67 ± 0.06 | 31.96 |
| Turmeric leaves | 12.67 ± 0.58 | 52.79 |
| Turmeric rhizome | 7.67 ± 0.58 | 31.96 |
| Torch ginger | 7.00 ± 0.00 | 29.17 |
| Ginger | 7.00 ± 0.00 | 29.17 |
| Galangal | 7.00 ± 1.00 | 29.17 |
| Tuhau | N | N |
| Clove | 14.97 ± 0.06 | 62.5 |
| Bay leaves | 9.00 ± 0.05 | 29.17 |
| Cinnamon | 24.33 ± 0.58 | 101.38 |
| Lemongrass | 10.17 ± 0.29 | 42.37 |
| Kaffir leaves | 7.00 ± 0.10 | 29.17 |
| Kaffir lime | 9.00 ± 1.00 | 37.5 |
| Star anise | 7.67 ± 0.58 | 31.96 |
| Garlic | N | N |
| Nutmeg | 7.67 ± 0.58 | 31.96 |
| Basil leaves | 7.67 ± 0.29 | 31.96 |
| Mint | 11.00 ± 0.50 | 45.83 |
| Rosemary | 8.00 ± 1.00 | 33.33 |
| Black pepper | N | N |
| Calamansi | 7.00 ± 0.00 | 29.17 |
| Tetracyline (1 mg/ml) | 24.00 ± 0.52 | |

N, no zone of inhibition was found. The percent of inhibition in compare to tetracycline

Table 3. Growth inhibition activity of *S.enterica* treated with essential oils extracted from herbs and spices

| Sample | Inhibition activity |
|---|---|
| Coriander seed | Inactive |
| Coriander leaves | Inactive |
| Cumin | Inactive |
| Fennel | Inactive |
| Cardamom | Inactive |
| Turmeric leaves | Active |
| Turmeric rhizome | Inactive |
| Torch ginger | Inactive |
| Ginger | Inactive |
| Galangal | Inactive |
| Tuhau | No activity |
| Clove | Active |
| Bay leaves | Inactive |
| Cinnamon | Potent |
| Lemongrass | Inactive |
| Kaffir leaves | Inactive |
| Kaffir lime | Inactive |
| Star anise | Inactive |
| Garlic | No activity |
| Nutmeg | Inactive |
| Basil leaves | Inactive |
| Mint | Inactive |
| Rosemary | Inactive |
| Black pepper | No activity |
| Calamansi | Inactive |

No activity 0% inhibition (no inhibition zone)
Inactive <50% inhibition
Active >50 – 59% inhibition
Moderate >60 – 79% inhibition
Potent >80% inhibition
The inhibition percentage was expressed in compared to positive control (tetracycline)

Table 4. Minimum inhibition concentration (MIC) of the most effective essential oils against *S. enterica*

| Essential oils | MIC (mg/mL) |
|---|---|
| Turmeric leaves | $3.13 \times 10^{-2}$ |
| Clove | $4.88 \times 10^{-4}$ |
| Cinnamon | $1.73 \times 10^{-4}$ |

Table 5. Comparison of antimicrobial activity of essential oils on different types of pathogen

| Essential oils | Antimicrobial activity (% inhibition) | | | |
|---|---|---|---|---|
| | Gram-positive | | Gram-negative | |
| | *S. aureus* | *L. innocua* | *E. coli* | *S. enterica* |
| Turmeric leaves | 57.58 | 27.66 | 65.67 | 52.79 |
| Clove | 42.42 | 42.33 | 65.67 | 62.5 |
| Cinnamon | 99.00 | No inhibition | 121.95 | 101.38 |

Note: Different inhibition zone for tetracycline between *E. coli* (27.33 ± 0.58), *S. aureus* (33.00 ± 1.00), *L. innocua* (38.58 ± 0.58) and *S. enterica* (24.00 ± 0.52)

However, no activity of cinnamon oils was observed on *L. innocua*. Clove essential oils was the most effective against *E. coli*, followed by *L. innocua*, *S. enterica* and *S. aureus*. Turmeric leaves were also effective against all four pathogens, especially against *E. coli*, followed by *S. aureus*, *S. enterica* and *L. innocua*. In general, essential oils that showed good inhibition against *S. enterica* also demonstrated a good antimicrobial activity against the other tested food-borne pathogens. The varying degrees of sensitivity of the bacterial test organisms may be due to both the intrinsic tolerance of microorganisms and the nature and combinations of phyto-compounds present in the essential oil (Aziz et al. 2018).

## Discussion

The development of food preservation processes has been driven by the need to extend the shelf-life of food products. Food preservation is a continuous fight against the microorganisms which make the food unsafe for consumption. In the production of food, it is important that proper measures were taken to ensure the safety and stability of the food products. Today, consumers are increasingly concerned about the food that they consumed. Consumers would prefer more high quality, chemical-free preservative, and safe processed foods with extended shelf-life. For this reason, there is a need to explore new alternative of food-grade antimicrobials in food preservation. Essential oils derived from spices and herbs have emerged as promising natural antimicrobials due to their diverse mechanisms of action, which help prevent microbial resistance. Additionally, they are generally recognized as safe for human consumption without limitations on intake, making them widely accepted by consumers.

Interestingly, the antimicrobial effects of cinnamon oils against *S. enterica*, were found to be as effective as tetracycline, the antibiotic used as positive control. Furthermore, cinnamon oils exhibited significant effectiveness against two other tested pathogen*, E. coli* (121.95%) and *S. aureus* (99%), although no activity was observed against *L. innocua*. The antimicrobial activity of cinnamon bark and oil has been supported by previous studies on *S. aureus* and *E. coli*; and against many bacteria fungi and even viruses such as *Candida albicans*, *Bacillus cereus, Salmonella typhimurium, Enteroccoccus faecalis, Klebsiella pneumoniae, Rhizomucor* sp.*, Pseudomonas* sp., *Proteus mirabilis*, *Streptococcus pyogenes, Colletotrichum coccodes*, *Botrytis cinerea*, *Cladosporium herbarum, Rhizopus stolonifer* and *Aspergillus niger* (Muthuswami et al. 2008; Tzortzakis 2009; Usha et al. 2012; Vazirian et al. 2015; D' Souza et al. 2017; Ramsey et al. 2020). Chemical analysis by Vazirian et al. (2015) identified five dominant compounds in cinnamon oils. These compounds were trans-cinnamaldehyde, linalool, cinnamaldehyde para-methoxy, eugenol and trans-caryophyllene at 79.73%, 4.08%, 2.66%, 2.37% and 2.05%, respectively. A strong antimicrobial activity of cinnamon essential oils has been reported in various studies in food and can be used individually or in combination with other essential oils. However, the effectiveness of cinnamon oils depends on their concentration and the types of food to which they are applied (Vazirian et al. 2015). Studies have demonstrated the bacterial inhibition activity of cinnamon oils when applied to cakes, does have bacterial inhibition activity on *E. coli*, *S. typhimurium*, *C. albicans* and *B. cereus* (Vazirian et al. 2015). The antimicrobial effect of trans-cinnamaldehyde emulsion on food was studied by Jo et al. (2015), and positive result was also found against *S.* typhimurium and *S. aureus.* These data on application of cinnamon oils and its chemical compounds does provide a good preservative activity when applied to food products without affecting the sensory evaluation. Nevertheless, further research is needed to fully establish its efficacy and safety for use in human.

Cloves are usually used for cooking, and ground cloves are used in baking breads/cakes and spice powder preparation. The data from this study demonstrates that clove is the second most effective spice against *S. enterica,* with a good minimum inhibitory concentration. Clove also shows high potential in inhibiting the growth of *E. coli*. However, the inhibitory activity of cloves against *S. aureus* and *L. innocua* was found to be lower than 50% compared to tetracycline, indicating that it is not as effective against gram-negative pathogens. Nevertheless, previous studies by Pandey et al. (2004) and Cui et al. (2018) have supported the antimicrobial activity of cloves against *S. aureus* and *L. monocytogenes*. Clove has also been found to inhibit other microorganisms such *K. pneumonia, L. monocytogenes, Aeromonas hydrophila, E. coli* O157:H7*, B. cereus, C. albicans, Streptococcus mutans, Lactobacillus acidophilus* and *Saccharomyces cerevisiae* (Pandey et al. 2004; D' Souza et al. 2017; Liu et al. 2017). The main active ingredients of cloves found are eugenol (eugenyl-acetate, methtyleugenol, dehydrodieugenol, and isoeugenol), β-caryophyllene, 2-heptanone, α-humulene, methyl-salicylate, phenylpropanoids, bioflorin, aldehydes, and oleanolic acid (D' Souza et al. 2017). According to Liu et al. (2017), components from clove possess the capability to disrupt the cell walls and membranes of microorganisms. Studies on *B. cereus* have demonstrated that eugenol can inhibit the production of amylase and protease and induce cell lysis (Liu et al. 2017). Similar to cinnamon oils, the safe concentration of clove oil needed to be established by safety evaluation.

Turmeric is indeed a widely recognised for its beneficial properties and extensively used as one of the ingredients in all *Ayurveda* systems (unprocessed plant origin medicines). Turmeric is known to contain curcumin, a dietary polyphenolic compound that have been shown to exhibit a potent antibacterial activity against various pathogenic bacteria, including *S. aureus*, *Streptococcus epidermidis, Bacillus subtilis, Bacillus macerans, Bacillus licheniformis, Enterococcus* and *Azotobacter* sp. (Rai et al. 2008; Naz et al. 2010; D'souza et al. 2017). Safety evaluation conducted by Chattopadhyay et al. (2004) have demonstrated that both turmeric and its purified compound, curcumin, are well-tolerated by the human body even at elevated doses. In Malaysian cuisine, turmeric leaves is commonly used as condiments such as in *rendang, masak lemak* and also used to wrap grilled fish. In the context of this study, turmeric leaves were found to possess effective antimicrobial properties against *S. aureus, E. coli* and *S. enterica,* although they were not as effective against *L. innocua*. The findings are supported by previous research by D' Souza et al. (2017) and Azhari et al. (2018), which also reported the antimicrobial effects of turmeric leaves against *S. aureus* and *E. coli*. Additionally, they also reported the antimicrobial activity against other pathogens, including *Shigella dysenteriae, B. macerans, B. licherniformis,* and *Helicobacter pylori* (D' Saouza et al. 2017; Azhari et al. 2018)

Regarding the antimicrobial activity of the three most effective essential oils against *S. enterica* in this study, it is evident that these oils are not as effective on gram-positive pathogens compared to the gram-negative. This difference in the effectiveness can be attributed to the distinct differences in their cell wall structure of gram-negative and gram-positive bacteria, which can influence their susceptibility to different antimicrobial agents based on their respective mechanism of action.

The antimicrobial impacts of essential oils and their chemical components have been recognised by several researchers in the past. However, it is challenging to determine whether the mechanism of action of these essential oils is through growth inhibition (bacteriostatic) or by destroying bacterial cells (bactericidal). They are usually screened by disc diffusion assay and measured by MIC or minimum bactericidal concentration (MBC) (Swamy et al. 2016). In order to successfully apply essential oils in various food systems, it is crucial to investigate the potential interactions between the essential oils and the components of the food. Numerous examples exist where certain studies have demonstrated the effectiveness of plant extracts in reducing pathogens in specific food products, while others have reported minimal antimicrobial activity or no impact when the same essential oils were applied to other products. As a result, the application of plant essential oils necessitates an evaluation of their efficacy within food products or in model systems that closely resemble the composition of the specific food. This is necessary because certain food components may diminish the effectiveness of the essential oils. However, it is also worth noting that essential oils are highly concentrated and should be use in caution. Ingestion of high doses of essential oils can be toxic and might cause serious adverse effects. Therefore, further research is needed to fully establish their efficacy and safety for use in food preservation. Determinations of the MIC of these essential oils is compulsory to help identify the lowest concentration of the agent that can effectively inhibit the growth of the microorganism. This information would be very useful in determining the optimum concentration of the antimicrobial for application with the potential to minimise any adverse effects associated with using high concentrations of essential oils.

## Conclusion

In conclusion, it can be generally concluded that these selected herbs and spices used in Malaysian cuisine have an effective inhibitory effect against *S. enterica* and other three food-borne pathogen tested, which could be considered as new alternative food grade antimicrobials for the preservation of foods. The varying degrees of sensitivity of may be due to the intrinsic tolerance of the pathogens. Essential oils from herbs and spices are the most promising natural antimicrobials because they do not cause microbial resistance due to the diversity of mechanisms of action. Furthermore, they are generally recognised as safe for human consumption without limitations on intake and commonly accepted by consumers. However, further study on its application on food products need to be evaluated since essential oils contains volatile aromas which might affects the taste or aroma of the food products.

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