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Review: Antimicrobial Properties of Common Herbs and Spices Used in Thai Cooking

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ABSTRACT

The common Thai cooking herbs and spices antimicrobial activities; Chilli (*Capsicum annuum*), Lemongrass (*Cympopogon citrates)*, Garlic (*Allium sativum*), Shallot (*Allium ascalonicum*), Galangal (*Alpinia galangal*), and Kaffir Lime (*Citrus hystrix*), have been reported numerously. Not only their antimicrobial activities but the antimicrobial active compounds and composition have been continuous reported under difference extraction methods and analysis methods. To understand antimicrobial mechanism of these herbs and spices will benefit to food industry. This article reviews the antimicrobial activities, the antimicrobial active compounds composition, the antimicrobial mechanism and the application in food of these herbs and spices.

Keywords: Chilli (*Capsicum annuum*), Lemongrass (*Cympopogon citrates*), Garlic (*Allium sativum*) Shallot (*Allium ascalonicum*), Galangal (*Alpinia galangal*), Kaffir Lime (*Citrus hystrix*), Antimicrobial, Cooking

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INTRODUCTION

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted [1]. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant [2]. These herbs and spices also have been used in cooking for long times all over the world. Nowadays, the use of natural antimicrobial as food preservation become more popular and more acceptable thus the consumption trends are changing. People concern about health and what they eat more. Thus from the globalization the international trading on foods and ingredients expand increasingly. Food safety has become an increasingly important international concern.

Antimicrobial referred to substances that can kill or inhibit the growth of the microorganisms [3]. Originally, an antimicrobial was a substance that produced by a microorganism that can inhibit or reduce the growth of another [4]. In addition, the synthetic antimicrobial are usually chemically related to natural antimicrobial which were produce to function in the same manner [4]. Thus Food is the ideal medium for the spread of harmful agents due to the ability of food to mask the harmful agents by strong flavors, strong odors, various textures or intense colors. Food and food ingredients are easily in distribution over great distances, there is increased potential for widespread impact from food and food ingredients [5]. Foodborne disease is an increasingly serious public health problem all over the world and the cause of that is determined to be microorganisms.

Spices and herbs have long been used in food for flavoring. Since the ancient times, they have been used for preventing food spoilage and deterioration and also for extending the shelf life of foods [6]. Thai food used a lot of herbs and spices in cooking to give signature flavor and taste. These herbs and spices; Chilli (*Capsicum annuum*), Lemongrass (*Cympopogon citrates)*, Garlic (*Allium sativum*), Shallot (*Allium ascalonicum*), Galangal (*Alpinia galangal*), and Kaffir Lime (*Citrus hystrix*), are common used in Thai food. These herbs and spices have been report for their antimicrobial activity.

CHILLI *(Capsicum annuum*)

Chilli *(C. annuum*) is a very good source of various vitamins and minerals such as vitamin C, vitamin B, vitamin B6, potassium, magnesium, and iron [7]. Also form Ethnobotanical data, *Capsicum* species harbor many potentially economically significant compounds yet to be discovered [8]. The plain and heated aqueous extracts from fresh *C. annuum* were tested for their antimicrobial effects with fifteen bacterial species and one yeast species [8]. The two pungent compounds found in *Capsicum* species (capsaicin and dihydrocapsaicin) were also tested for their antimicrobial effects [8]. The plain and heated extracts were found to exhibit varying degrees of inhibition against *Bacillus cereus, B. subtilis, Clostridium sporogenes, C. tetani*, and *S. pyogenes* [8]. It was investigated that the main chemical component was capsaicin [9]. Capsaicin is a hydrophobic molecule with boiling point of 210-220°C [9] with broadly antimicrobial activities on both bacterial and fungal such as *Fusarium* [10], *Helicobacter pylori* [11], *Botrytis cinerea*, and *Aspergillus niger* [12]. It showed that by adding 1% w/v of dried chili in BHI can slightly inhibited the growth of *Listeria monocytogenes* [13]. Hence by increasing the amount of chili might increase the inhibition activity [13]. In order to explore the inhibitory effect of the *C.annuum*, as a possible alternative to antibiotics against the challenge dose of *Salmmonella typhimurium* in broiler chickens were studied [14]. The results showed that the use of mixed diet with *C. annuum* at percent 1% and 2%, were effective against *S. typhimurium* infection through decreasing fecal shedding, isolation rate, bacterial count of *S. typhimurium* [14].The dry chili extract under Kang-Kati using fresh coconut milk extraction condition can inhibit *L. monocytogeneses, S. enterica* Enteritidis (human) and , *S. enterica* 4,5,12:i:- (human) US clone *[15].*

The inhibitory effects of the ethyl acetate extract and capsaicin and dihydrocapsaicin isolated from fruits of *C. annuum* , and synthetic capsaicinoid derivatives (*N*-(4-hydroxyphenylethyl)decamide, (*E*)-*N*-(4-hydroxy-3-methoxybenzyl)-3,7-dimethylocta-2,6-dienamide, 4-hydroxy-3-methoxy-*N*-((*E*)-3,7-dimethylocta-2,6-dienyl)benzamide, and *N*-(4-hydroxy-3-methoxybenzyl)decamide at different concentrations were evaluated against *Streptococcus mutans [16].* The minimum inhibitory concentration at which the ethyl acetate extract prevented the growth of *S. mutans* was 2.5 mg/mL; those of the isolated capsaicin and dihydrocapsaicin were 1.25 µg/mL, while synthetic capsaicinoid derivatives ; (N-(4-hydroxyphenylethyl)decamide was 5.0 µg/mL, and (E)-N-(4-hydroxy-3-methoxybenzyl)-3,7-dimethylocta-2,6-

dienamide, 4-hydroxy-3-methoxy-N-((E)-3,7-dimethylocta-2,6-dienyl) benzamide and N-(4-hydroxy-3-methoxybenzyl)decamide were 2.5 µg/mL, respectively *[16].* The antimicrobial activities of the ethyl acetate, acetone and methanol extract of *C. annuum were tested in vitro against 2 fungi and 8 bacterial species by the disc diffusion method* [17]. *The methanol extracts did not inhibit microorganisms tested except for Pseudomonas aeroginosa ATCC 27859* [17]*. While the acetone extracts showed antibacterial activity P. aeruginosa ATCC 27859 and Rhodotorula rubra, the ethyl acetate extracts showed antibacterial activity P. aeruginosa ATCC 27859 and Klebsiella pneumonia 13883* [17]*.*

It has become increasingly clear in antimicrobial mechanism that peptides play an important role in the protection of plants against microbial infection. The proteins from C.annuum seeds were extracted in phosphate buffer, pH 5.4 and peptides purification were performed by employing ion-exchange chromatography on DEAE, CM-Sepharose, Sephacryl S-100 and reverse phase in HPLC *[18]*. The 3 peptide enriched fractions, namely F1, F2 and F3, were obtained after the CM-Sepharose chromatography *[18]*. The F1 fraction, mainly composed of three peptides ranging from 6 to 10 kDa, exhibited strong fungicidal activity against Candida albicans, Saccharomyces cerevisiae and Schizosaccharomyces pombe and also promoted several morphological changes to *C. albicans,* including the formation of pseudohyphae, as revealed by scanning electron micrography *[18]*. The proteins from C.annuum's seed flour were extracted in phosphate buffer, pH 5.4, for 3 h at 4 °C. One of the resulting fractions, named F3, enriched with basic proteins of 6–16 kDa, inhibited the growth of yeasts *S. cerevisiae, C. albicans, C. parapsilosis, C. tropicalis, Pichia membranifaciens, Kluyveromyces marxiannus and C. guilliermondii* [19]*.*

LEMONGRASS (*Cympopogon citratus*)

Lemongrass (*C. citrates*) is common used in Thailand, Vietnam, Cambodia and Indonesia cooking. In Thailand, finely ground fresh lemongrass is added to curry pastes. The previous study also reported that the major chemical constituents of *C. citratus* from chemical analysis, GC analysis, of lemon grass oil contains geranial (citral a) about 28.93% and neral (citral b) about 18.49%, respectively [20]. Lemongrass oil contains citral at concentrations of approximately 65–85% w/w [21].

Out of the one hundred and fourteen strains belonging to twenty-nine genera and one hundred and five species of microbes (molds, yeasts and bacteria) isolated from different sources; clinical cases, environment (water, air, soil, droppings of lizards and birds), food and healthy animals, 38.2% were sensitive to lemongrass oil discs containing 50 µg oil/disc [22]. All molds, yeasts, *Lactobacillus acidophilus*, *Morganella morganii*, most of the *Bacillus* spp. strains (84.3%), *aromonads* (78%), *Edwardsiella* spp. (73.9%), 53.6% *pseudomonads*, 53.1% *streptococci* and 50% of *Budvicia aquatica* and *Leminorella ghirmontii* strains were sensitive to lemongrass oil [22]. On the other hand, all *Hafnea alvei, Laclercia adecarboxylata, Xenorhabdus luminescens* and majority of *S. enterica* (98.3%), *Citrobacter* spp. (93.7%), *Providencia* spp. and *Kluyvera cryocrescens* (83.3%), *Enterobacter* spp. (78.2%), *Proteus* spp. (78%), *Escherichia* spp. (77.7%), *enterococci* (73.7%), *Serratia* spp. (75%) and *Erwinia ananas* (75%), *Pragia fontium* (70.6%), *staphylococci* (69.8%) and *Klebsiella* spp. (62.7%) strains were resistant to lemongrass oil [22].

The inhibition of the chloroform leaf and corresponding root extracts of lemongrass for the test organisms were *Staphylococus aureus* ;11.33±1.15,11.66±2.52 mm, *S. typhi*; 11.33±1.53,13.66±0.58 mm, *Escherichia coli*; 16.33±0.58,15.66±2.31 mm and *C. albicans* ;7.66±0.58,8.66±1.53mm, respectively [23]. Hexane and methanol extracts showed no activity against the test microorganisms [23]. The minimum inhibitory concentration (MIC) and the corresponding minimum bactericidal concentration (MBC) for chloroform leaf and root extracts were : *S. aureus* (24µg/ml, 28µg/ml), *S. typhi* (20µg/ml, 28µg/ml), *E. coli* (14µg/ml, 16µg/ml), *C. albicans* (32µg/ml, 38µg/ml) and *S. aureus* (20µg/ml, 26µg/ml), *S. typhi* (18µg/ml, 24µg/ml), *E. coli* (14µg/ml, 16µg/ml), *C. albicans* (28µg/ml, 32µg/ml) respectively [23].

The ethanolic and aqueous extracts of lemongrass were tested using the agar well diffusion method to evaluated antimicrobial activity [24]. The test bacteria were*; S. aureus, E. coli, B. subtilis, Micrococcus* sp, S*. typhimurium, P. vulgaris, P. aeruginosa* and the test fungi were; *A. niger, A. flavus, Penicillium notatum, Trichophyton rubrum, C. albicans, Trichoderma viridae* and *Rhizopus nigricans* [24]. The ethanolic extract was effective against *S. aureus* (4.75mm), *E. coli* (5mm), *B. subtilis* (7.5mm), *Micrococcus* sp.(8mm), *T. rubrum* (15.5mm) and *C. albicans* (8.5mm) [24]. The ethanolic extract was inactive on *S. typhimurium, P. vulgaris, P. aeruginosa, A. niger, A. flavus, P. notatum, T. viridae* and *R. nigricans* [24]. The aqueous extract was effective

against *S. aureus* (5mm), *B. subtilis* (6.75), *Micrococcus* sp (7.25mm), *T. rubrum* (8.5mm) and *C. albicans* (7mm) while it was inactive on *E. coli, S. typhimurium, P. vulgaris, P. aeruginosa, A. niger, A. flavus, P. notatum, T. viridae* and *R. nigricans* [24]. The MIC of the ethanolic extract ranged from 0.25mg/ml to 0.5mg/ml while it was 0.25mg/ml for the aqueous extract [24]. The MBC of the ethanolic extract ranged from 0.5mg/ml to 1mg/ml while it was 0.5mg/ml for the aqueous extract [24]. The ethanolic extract was generally more active than the aqueous extract [24]. The ability of these extracts of lemongrass to inhibit the growth of bacteria and fungi is an indication of its broad spectrum antimicrobial potential which may be employed in the management of microbial infections [24].

The extracts was screened against two gram-negative bacteria, *Klebsiella pneumoniae* and *P. vulgaris*, two gram-positive bacteria, *B. subtilis* and *S. aureus* and fungi strain *Penicillium* and *Mucor* at three different concentrations [1:1, 1:2 and 1:3] using Disc diffusion method [25]. The ethanol extracts showed appreciable both antimicrobial and antifungal activity against *S. aureus*, *P. mirabilis*, *K. pneumonia* and *C. albicans* with MIC of 0.78mg/ml [26]. Both aqueous and ethanol extract of lemongrass extracts, by disc diffusion method, were found to exhibit selective inhibition against the isolates [27]. Ethanol extract exhibited high inhibitory activity against all the tested bacteria in order of sensitivity as *S. aureus, S.typhi, B. aureus, E. coli*, while aqueous extract was more active against *S. typhi* [27].

Five main food-borne pathogens inclus-ding *S. aureus, E. coli, C. albicans, B. cereus* and S. *typhimurium* were added to cream-filled cakes and lemongrass essential oil showed potent antimicrobial activity against selected microorganisms [28]. Minimum inhibitory concentration (MIC) values for essential oil against all tested microorganisms were determined as 0.5 µL/disc except for *S. aureus*, in which the oil was ineffective [28]. By using 1 µL/mL of essential oil, more than a 99.9% reduction in susceptible microorganisms was observed [28]. After baking, the cream-filled cake with four main susceptible pathogens manually added, after 72 hours of baking, no observable microorganism was observed [28]. The essential oil of lemongrass demonstrated bacterial activity at all concentrations (0.5, 1.5, 2.5, 5.0, 10.0, 15.0, 25.0, and 50.0%) and against all of the bacteria; *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *L. monocytogenes* ATCC 19117, *S. enterica* Enteritidis S64, and *P. aeruginosa* ATCC 27853 [29]. The Gram-negative bacteria demonstrated higher resistance to the use of the essential oils tested in this study. *E. coli* was the least sensitive and was inhibited only by the oils of *C. citrates* [29]. The antimicrobial potentials of essential oils, water extract, and freeze dried extract from the leaves of lemongrass were tested on *S. typhi, S. aureus* and *E. coli*, Lemon grass oil was observed to possess high antimicrobial activity on all the three bacteria tested [30]. Both the freeze dried extracts and the viscous extracts possess slight antimicrobial activity, the aqueous extracts have no effects on the bacteria [30].

Phytochemical screening on lemongrass showed that five active ingredients: Tannins, Flavonoids, Phenols, Carbohydrates and volatile oil were present in both the root and leaf parts [31]. The inhibition showed that lemongrass exhibited an intermediate antimicrobial activity against the bacteria species while *C. albicans* was resistant [31]. Higher dose of C. citratus may be recommended to exert a remarkable antimicrobial activity against the test organisms [31]. The essential oils from leaves of C. citratus from Burkina Faso were analyzed by GC-FID and GC-MS [31]. Five constituents, which accounted for 96.3% of the oil, were identified in the essential oils of lemongrass. Geranial (48.1%), neral (34.6%) and myrcene (11.0%) were the major constituents [31]. The dominant compounds were limonene (42%) and a set of monoterpene alcohols: trans-p-mentha-1(7),8-dien-2-ol (14.2%), cis-p-mentha-1(7),8-dien-2-ol (12%), trans-p-mentha-2,8-dien-1-ol (5.6%) and cis-p-mentha-2,8-dien-1-ol (5.2%) [31]. For both the leaves and stem extracts, the phytochemical analysis revealed the present of tannins, flavonoid, phlobotannins and cardiac glycosides but absence of alkaloid and saponin [25]. The phytochemical investigation revealed the presence of flavonoids, anthraquinones, alkaloids, saponins, phenols and steroids [32]. Phosphorus was found to be the most abundant (15.58mg/100g) followed by Potassium (8.60mg/100g) [27]. Zinc an important microelement was present inconsiderable amount (0.93mg/100g). Phytate and Oxalate contents were 0.48±0.02 and0.48±0.05mg/g respectively [27]. Terpenoid, Cardiac glycosides and Phenol were also present [27]. The chemical composition of the oil was analyzed by GC/MS and 15 components were identified, where neral (39.0%), geranial (33.3%), limonene (5.8%) and geranyl acetate (4.2%) were the most abundant constituents [28]. The majority of lemongrass essential oil compounds were geranial and neral using gas GC/MS [29]. The phytochemical analysis of the ethanolic extracts of cymbopogon citratus indicates that it has alkaloids, saponins tannins, anthraquinones, steroids, phenols and flavonoids [33].

GARLIC (*Allium sativum*)

Garlic (*A. sativum*) is an important food ingredient for many countries, especially in Asia. It is one of the herbs that have a lot of scientific report about its antibacterial and antifungal properties which comes from the substance called allicin (allyl 2-propene thiosulphinate) [34-37]. Alicin inhibits various thiol-dependent enzymatic systems of bacteria [38]. It is one of the active ingredients found during crushing garlic [38]. It reported the use of 5.5% v/v garlic oil and 12.5–25% v/v garlic powder to completely inhibit the growth of *S. enterica* at 37̊C [39]. It was noticed 2-log reduction in *S. enteric*a concentration with 1% v/v garlic oil at 37̊C [40]. In term of fresh garlic [41] found that raw garlic extract is a more effective antimicrobial agent than antibiotics currently in use; Ciprofloxacin and Ampicillin when testing on *Salmonella* spp. Also the effect of garlic extract is most pronounced on enteric bacterial pathogens. Garlic was found to be effective against *L. monocytogenes* [42,43]. There was the study of the effect of raising the temperature on the effectiveness of garlic, it was that found that the activity of garlic increased with increase in temperature up to 80̊C, beyond which the activity remained constant or decreased [44,45].

Allicin was proved that they can inhibit the growth of *E.coli*, *Shigella* sp., *Salmonella* sp., *P. mirabilis*[41], *K. pneamoniae* [46] , *Aeromonas caviae, A. hydrophila, A. sobria, Chromobacterium violaceum, Enterobacter faecalis, P. aeruginosa, B. subtilis* and *S. aureus* [44, 48] by partially inhibiting DNA and protein synthesis and also totally inhibiting RNA synthesis as a primary target [36,37,47]. In addition, they also has antimycotic properties on *A. fumigatus* and *A. niger* by hydro distilled method[48]. However, allicin will not present unless the barriers of enzyme alliin alkyl-sulfenate-lyase (EC 4.4.1.4) and non-protein amino acid S-allylcysteine S-oxide (alliin) were broken-down [35, 37], or only generated after the cloves are injured and the enzyme alliinase reacts with its substrate alliin [49]. The effectiveness of the garlic extract was proof that it is increased as the temperature increase, the optimum temperature is about 80^{O}C, aqueous extraction, and decreased or maintain after this range of temperature, because the higher temperature will increase the solubility of the chemical compounds[36, 44]. However, comparing between the dried and fresh form, it showed that fresh extracts had more antimicrobial properties than dried autoclaved extracts[50]. The antibacterial activity was tested against *B. subtilus* (DSM 3256) and *E.coli* (ATCC 25922) at different concentration of extracts of spices by using disc diffusion method [51]. According to the results among the selected spices garlic had the best inhibitory activity showing maximum zone of 26 mm. against *B. subtilis* DSM and a zone of 22 mm. Against *E.coli* ATCC 25922 [51]. The aqueous extracts of garlic were more effective than ethanolic extract [51]. The anti-microbial activity of a range of garlic products including dried garlic powder produced by different methods, commercial garlic products, and garlic oil was determined against a range of selected bacteria; *S. aureus, E. coli, S. typhimurium, B. cereus*, and a mixed lactic culture consisting of *L. delbrueckii* subsp. Bulgaricus and *S. thermophilus* [52]. Generally fresh garlic produced the greatest inhibition followed by freeze-dried powder [52]. The results showed that both drying temperature and time had major effects on retaining the active components responsible for the inhibition of microbial growth [52]. Allicin showed antibacterial effect against *H. pylori* [53]. There was studiy the inhibitory effect of garlic extract with allicin on oral bacteria including 13 gram-positive and 6 gram-negative types of bacteria, and one fungi [54]. The garlic extract (57.1% (w/v), containing 220µg/ml allicin) inhibited the growth and killed most of the organisms tested [54]. In general, the minimal inhibitory and minimum bactericidal concentrations for the Gram-negative strains (garlic MIC range 35.7–1.1mg/ml; allicin mean MIC 4.1µg/ml; mean MBC 7.9µg/ml) were lower than those for the Gram-positive strains tested (garlic MIC range142.7–35.7mg/ml; allicin mean MIC 27.5µg/ml; mean MBC 91.9µg/ml) [54]. Also, of the organisms tested, the putative periodontal pathogens had among the lowest MICs (17.8–1.1mg/ml garlic) and MBCs (35.7–1.1mg/ml garlic) [54].

The mode of action of aqueous garlic extract was studied in *C. albicans* [55]. The anticandidal activity of aqueous garlic extract was antagonized by thiols such as L-cysteine, glutathione and 2-mercaptoethanol. Interaction studies between aqueous garlic extract and thiols included growth antagonism, enzymic inhibition and interference of two linear zones of inhibition [55]. All three approaches suggest that aqueous garlic extract exerts its effect by the oxidation of thiol groups present in the essential proteins, causing inactivation of enzymes and subsequent microbial growth inhibition [55]. Allicin is shown to be a specific inhibitor of the acetyl-CoA synthetases from plants, yeast and mammals [56]. The bacterial acetyl-CoA-forming system, consisting of acetate kinase and phosphotransacetylase, was inhibited too. Non-specific interaction with sulfhydryl-groups could be excluded in experiments with dithioerythritol and p-hydroxymercuribenzoate [56]. Binding of allicin to the enzyme is non-covalent and reversible [56]. The (14C)-Acetate incorporation into fatty

acids of isolated plastids was inhibited by allicin with an I50-value lower than 10 µM [56]. Other enzymes of the fatty acid synthesis sequence were not affected, as was shown using precursors other than acetate [56].

Ajoene, a garlic-derived sulfur-containing compound that prevents platelet aggregation, exhibited broad-spectrum antimicrobial activity [57]. Growth of gram-positive bacteria, such as *B. cereus, B. subtilis*, *Mycobacterium smegmatis*, and *Streptomyces griseus*, was inhibited at 5 mg ajoene/ml. *S.aureus* and *L. plantarum* also were inhibited below 20 mg ajoene / ml [57]. For gram-negative bacteria, such as *E. coli*, *K. pneumoniae*, and *Xanthomonas maltophilia*, MICs were between 100 and 160 mg/ml [57]. Ajoene also inhibited yeast growth at concentrations below 20 mg/ml [57]. The microbicidal effect of ajoene on growing cells was observed at slightly higher concentrations than the corresponding MICs. *B. cereus* and *S. cerevisiae* were killed at 30 ml ajoene / ml after 24 hr of cultivation when cultivation was started at 10^5cells per ml [57]. The disulfide bond in ajoene appears to be necessary for the antimicrobial activity of ajoene, since reduction by cysteine, which reacts with disulfide bonds, abolished its antimicrobial activity [57].

The Six different mixtures of garlic distilled oils containing diallyl disulfide and diallyl trisulfide, ranging from 1 to 51 % and 88 to 38 % respectively, have been assayed against a number of yeasts (*C. albicans, C. tropicalis* and *B. capitatus*), Gram-positive bacteria (*S. aureus* and *B. subtilis*) and Gram-negative bacteria (*P. aeruginosa* and *E. coli*) [58]. The results support a specific antifungal more than an antibacterial activity and implicate garlic distilled oils containing diallyl disulfide as the active constituent [58]. Incubation of garlic extracts made up of 1 % garlic distilled oils containing diallyl disulfide and 88 % garlic distilled oils containing diallyl trisulfide resulted, in fact, in the absence of growth inhibition against all the tested microorganisms, whereas garlic oils with higher quantities of garlic distilled oils containing diallyl disulfide showed significant inhibitory activity, increasing with the increase of garlic distilled oils containing diallyl disulfide amount [58].

SHALLOT (*Allium ascalonicum*)

Shallot (*A. ascalonicum*) is the herb that has many biologically active compounds of antimicrobial properties which included flavonoids and phenolic acids [59]. The flavanols that found in shallot are quercetin and kaempferol. However, it was also proof that the bulbs part contains only quercetin, but the leaves part contains both quercetin and kaempferol [59]. The main active compound of *A. ascalonicum* L. (Shallot), flavanols and phenolic compounds [59], have broad spectrum against both fungal and bacterial such as *Syncephalastrum*, *A. niger*, *Penicillium* sp., *Paecilomyces* sp., *Scopulariopsis* sp.[60-61], *B. cereus*, *E. coli* O157:H7, *S. enterica* [62], *B. cereus* and *S. aureus* [63]. Shallot oils significantly inhibited the growth of 4 food-borne bacteria, S*. typhimurium DT104, E.coli O157:H7, L. monocytogenes* and *S. aureus*, and 4 nosocomial bacteria, methicillin-resistant *S. aureus, K. pneumoniae, P. aeruginosa* and *Acinetobacter baumannii* ($p < 0.05$) [67]. The shallot ethyl acetate extract could be used as an effective antibacterial agent against *M. tuberculosis,* which is a resistant infection in pulmonary tuberculosis [68]. The shallot extract showed antimycobacterial activity with a minimum inhibitory concentration (MIC) value of 500µg/ml [68].

Extract of shallot only was soluble in dimethyl sulphoxide, dimethyl formamide and water which means that the compound present in the shallot is polar compounds [61]. However, the previous study also indicated that the oil extraction method provide a better antibacterial activity than the fresh extraction by manual extractor and squeezing [63]. Fresh extract of garlic showed greater antimicrobial activity as compared to similar extracts of onion and shallot. However, dried and autoclaved extracts of shallot showed more activity than similar extracts of onion and garlic [64]. Antimicrobial compound of water extract of shallot was very heat stable and active over a wide range of pH [61]. Antimicrobial principle of shallot was resistance to and active under both acid pH and basic pH (pH 4-8) [61]. Different temperatures did not show significant effect on antimicrobial activity of shallot extract [61]. These results increase the chance of utilization of shallot extract as a natural preservative at different temperatures from -7 °C - 121°C [61]. The hydroalcoholic extracts of shallot showed stronger effects on 15 isolated vancomycin resistant *S. epidermidis* [70]. The shallot under three extraction methods ; (Kaeng Kathi - oil using fresh coconut milk, Kaeng Kathi - oil using UHT coconut milk, and Kaeng Pa - water) showed antibacterial against *S.* Typhimurium DT104b [72].

The effects of shallot extract on the quality of vacuum-packaged rainbow trout (*Oncorhynchus mykiss*) were examined during refrigerated storage (4 ± 1 °C) over a period of 20 days [69]. The results indicated that the effect of the shallot extract on the fish samples were to enable the good quality characteristics to be retained longer and to extend the shelf life during the refrigerated storage [69].

An isolation procedure comprising ion exchange chromatography on DEAE–cellulose, affinity chromatography on Affi-gel blue gel, ion exchange chromatography on SP–Sepharose and gel filtration on Superdex 75 was used to isolate an anti-fungal peptide from the bulbs of the shallot (*A. ascalonicum*) [66]. The peptide demonstrated a molecular weight of 9.5 kDa, and possessed an N-terminal sequence YQCGQGG somewhat similar to chitinases from other Allium species which are however much larger in molecular weight [66]. The peptide designated ascalin manifested a unique specific anti-fungal activity. It inhibited mycelial growth in the fungus *Botrytis cinerea* but not in the fungi *Mycosphaerella arachidicola* and *Fusarium oxysporum* [66]. The Biochemical analysis by GC-MS show that presence of 13 substances (80.3 %) mainly including organosulfur in *A. ascalonicum* essential oils [71]. The 2000 ppm *A. ascalonicum* essential oils during ripening period of Iranian White Brined Cheese had the highest decrease in the bacterial colony counts compared to other treatments ($P< 0.05$) but cheese organoleptic assessment demonstrated that 750 ppm *A. ascalonicum* essential oil maintained the highest acceptable range [71].

GALANGAL (*Alpinia galangal*)

Galangal (*A. galangal*) can be also used not only for food ingredients but also for medical purposes, such as forcarminative, stomachic, antispasmodic, antichloristicand antibacterial drugs[72,73]. The main components of the galangal's oils were also tested and terpinen-4-ol was found most active as antifungal [74]. An n-pentane/diethyl ether extract of dried rhizomes was active against *Trichophyton mentagrophytes* [74]. The active compound that contains in the galangal was proof that it will provide a highest antimicrobial affect with the non-polar extraction by the non-polar extractant such as hexane [75]. By the scanning electron microscopy observations, it showed that galangal's oil has a mechanism that can modify of the bacterial cell membrane, disrupting the membrane's permeability which make the spill out of cell materials and the death of pathogens cell [76]. The previous study al so showed that the extract from *A. galangal* has antimicrobial activity on many microorganisms such as *S. typhimurium*, *S. aureus*, *F. solani*, *B. cinera* KCTC 6973[77], and *L. monocytogenes*[75]. *S. aureus* was more sensitive to the ethanol and hexane extracts than *E.coli* and *S. typhimurium* [77]. Galangal extract at higher concentrations of 0.05% and 0.10% (wt/wt) were also found to extend the shelf-life of minced beef [80]. It indicated that the essential oil of galanga had the bactericidal activity [77]. However, the previous study also indicated that the oil extraction method provide a better antibacterial activity than the fresh extraction by manual extractor and squeezing [63]. The galangal extract had thestrongest inhibitory effect against *S. aureus* [78]. The MIC of the galangal extract was 0.325 mg/ml and the MBC at 1.3 mg/ml using the broth dilution method [78]. But *S. enterica* Typhimurium and *E. coli* O157:H7 were resistant to alangal ethanol extracts [83].

Crude extract of galangal was the lipophilic compounds, soluble in ethanol which is a property of essential oils [78]. Most major and minor compounds were identified by GC-MS as ACA, *p*-coumaryl diacetate, palmitic acid, acetoxyeugenol acetate, eugenol, β-bisabolene, β-farnesene and sesquiphellandrene were the phenolic compounds, phenolic derivative compounds, the ester of weak acid, fatty acid, terpenes and others [78]. Transmission electronmicroscopy clearly demonstrated that the galangal extract caused both outer and inner membrane damage, and cytoplasm coagulation [78]. There were large number of different chemical compounds presented in galangal crude extract therefore its mechanism of action can affect multiple target sites against the bacterial cell [78]. The β-bisabolene, β-farnesene and sesquiphellandrene are terpenes in the essential oils of spices that mechanism of action should be similar to other terpenes and other phenolic compounds in this crude extract as indicated as involving disruption of the cytoplasmic membrane and coagulation of cell contents [78]. The essential oils are hydrophobicity which enables them to a partition in the lipids of the bacterial cell membrane disturbing the structures and rendering them more permeable so the galangal extract affected the cytoplasmic membrane of *S. aureus* and induced the loss of nucleic acids and ions [78]. ACA; main compound in this crude extract, is ester of acetic acid that its meachanism is having membrane gradient neutralization and denaturing of proteins inside the cell [78]. Galangal extract was added to the edible chitosan film forming solution as a natural antimicrobial agent in the concentration range of 0.3–0.9 g/100 g [82]. The antimicrobial activity, swelling and functional group interaction of the antimicrobial films were found to be affected by the drying methods and conditions as well as the concentration of the galangal extract [82]. The electron microscopic observations revealed that cell wall and cell membrane of *S. aureus* treated by the antimicrobial films were significantly damaged [82].

The synergistic antimicrobial activities of combinations of galangal with either rosemary or lemon iron bark showed synergistic antimicrobial activity. Specifically, galangal and rosemary showed synergistic activity

against *S. aureus* and *L. monocytogenes* only, while galangal and lemon iron bark showed synergistic activity against *E. coli* and *S. typhimurium* [79]. The major chemical components of the galangal extracts were 1'-acetoxy-chavicol acetate (1'ACA) (63.4%) [79]. The galangal-hoan ngoc extract also showed the synergistic antimicrobial activities of combinations againsted *Campylobacter* spp [81].

KAFFIR LIME (*Citrus hystrix*)

There are numberous reports in kaffir lime's (*C. hystrix*) antimicrobial activity. The crude ethnolic extracts of kaffir lime peel showed the broadest antibacterial activity by inhibiting growth of 20 serotypes of *Salmonella* and 5 species of other enterobacteria including *S. Agona, S. Anatum, S. Choleraesuis, S. Derby, S. Enteritidis, S. Lexington, S. London , S. Newport, S. Newport, S. Senftenberg , S. Virchow , S. Weltevreden , S. Typhimurium DT104, S. Typhimurium non-DT104, S. Amsterdam, S. Hardar, S. Orion, S.panama S. Schwarzengrund , S. Stanley, C. freundii, E. aerogenes , E. coli, K. pneumoniae , and S. marcescens* [84]. While the crude ethnolic extracts of kaffir lime leave also showed the broadest antibacterial activity same as the crude ethnolic extracts of kaffir lime peel, expect only *S. marcescens* [84]. The essential oils of kaffir lime peel against all 20 serotypes of *Salmonella* and 5 species of other enterobacteria while the essential oils of of kaffir lime leave aginast all most tested microorganisms expect *S. Newport, S. Newport , S. Newport,* and *E. aerogenes* [84]. In general, the inhibitory activity of essential oils was greater than that of ethanolic extracts, especially in kaffir lime peels [84]. Among the serotypes of *Salmonella* tested, the ethanolic extracts and oils exhibited slightly different degree of inhibition. *S. Typhimurium* (non-DT104 strain) was the most susceptible serotype to both oils and extracts of kaffir lime's peels [84]. The use of pressurized hot water extraction on kaffir lime fruit peel and found out that when increase temperature in extraction the phenolic compound content increasing [85]. The ethyl acetate extract of kaffir peel showed broad spectrum of inhibition against all Gram-positive bacteria, yeast and molds including *S. aureus, B. cereus*, *L. monocytogenes, S. cerevisiae* var. sake and *A. fumigatus* TISTR 3180 [87]. The kaffir lime leaf oil and kaffir lime peel oil were both effective against all the pathogens were both effective against all the pathogens including 411 clinical isolates obtained from patients with respiratory tract infections ; *A. baumannii* (50 isolates), Groups A (61 isolates), B (27 isolates), C (4 isolates), F (3 isolates), G streptococci (11 isolates), *Haemophilus influenzae* (52 isolates), *M. catarrhalis* (52 isolates), methicillin-resistant *S. aureus* (MRSA; 50 isolates), methicillin sensitive *S. aureus* (MSSA; 50 isolates) and *S. pneumoniae* (51 isolates) [90]. The crude ethanolic extracts of kaffir lime leaf at 10 % showed significantly higher inhibition than at other concentrations against *A. flavus* [91]. The kaffir lime leaf essential oils showed the strongest antibacterial activity with inhibition zones of more than 20 mm against all 5 strains of *Propionibacterium acnes* (DMST No. 14916, 14917, 14918, 21823, 21824) [65]. The kaffir lime essential oils also showed antibacterial activity against all 5 strains of *P.acnes* as well [65].

For antimicrobial activity, 10% kaffir lime peel essential oil could extend shelf-life of Chinese sausages by 5 days, when compared to the control (without essential oil addition) [89]. Another food application is the antibacterial rinse. It was formulated as an emulsion, consisted of 40% v/v Kaffir lime oil, 8% w/v gelatin, and 3% w/v lecithin [86]. The emulsion was diluted with water into soaking solution which contained 0.75% v/v of Kaffir lime oil [86]. The soaking solution reduced the natural bacterial population on chinese cabbage, by means of aerobic plate count after the second water rinse, by 2.68, 3.30 and 4.27 log at 5, 10 and 15 min soaking time, respectively [86].

The volatile oil kaffir lime peel was analyzed, using GC-MS. The major constituents were l-limonene, a-terpineol, 2-b-pinene, terpinene-4-ol, g-terpinene, a-terpinene, and a–terpinolene [86]. Another work also showed the major components of the ethyl acetateextract from kaffir lime were limonene (31.64 %), citronellal (25.96 %) and β-pinene (6.83 %) whereas β-pinene (30.48 %), sabinene (22.75 %) and citronellal (15.66 %) appeared to be major compounds of the essential oil obtained from hydrodistillation [87]. The kaffir lime peel essential oil chemical composition, was analysed by GC-MS, consisted of several components (limonene 40.65%, terpinene-4-ol 13.71%, α-terpineol 13.20%), and the most active component was α-terpineol, followed by terpinene-4-ol, and limonene [90]. Fresh kaffir lime leaves were subjected to extraction by supercritical carbon dioxide yielding yellow clear oils which were then analyzed by gas chromatographic-mass spectrometric technique (GC-MS) [92]. Results from GC-MS analysis revealed 21 identified terpenoids which were classified in the groups of monoterpenes, oxygenated mo noterpenes, sesquiterpenes and oxygenated sesquiterpenes, each having the number of 3, 5, 9, and 4, respectively. Citronellic acid (4.5%), nerolidol (2.14%), δ-cadinene (1.49%), citronellal (1.41%) and citronellol (1.39%) were found as the major constituents [91].

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