International Journal of Microbiology Research and Reviews ISSN 2329-9800 Vol. 8 (1), pp. 001-007, January, 2019.
Available online at www.internationalscholarsjournals.org © International Scholars Journals

Author(s) retain the copyright of this article.

*Full Length Research Paper*

# Essential oil composition and antibacterial activity of *Zanthoxylum bungeanum*

**Rui-Xue Zhu[1], Kai Zhong[1], Wei-Cai Zeng[1], Xue-Yun He[2], Xue-Quan Gu[2], Zhi-Feng Zhao[1]* and Hong Gao[1]**

[1]College of Light Industry, Textile and Food Engineering, Sichuan University, Chengdu 610065, P. R. China.
[2]Sichuan Wufeng Lihong Food Co., Ltd., Ya'an, 625302, P. R. China.

Accepted 19 September, 2018

**The chemical composition and antibacterial activity of essential oil from the fruits of *Zanthoxylum bungeanum* were evaluated. Twenty-eight compounds were identified by gas chromatography-mass spectrometry (GC-MS) analysis, representing 94.30% of the total oil. The major constituents of the essential oil were found to be limonene (29.36%), linalool (17.02%), β-pinene (14.31%) and linalyl acetate (13.84%). The essential oil showed an obvious antibacterial activity against the tested strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus laterosporus laubach* and *Escherichia coli*) with the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in the ranges of 1.25-5.0 and 2.5-20 mg/ml, respectively. Furthermore, the essential oil showed strongly detrimental effects on the growth and morphological structure of the tested bacteria. So, it is suggested that *Zanthoxylum bungeanum* could be used as antibacterial agent in food industry.**

**Key words:** *Zanthoxylum bungeanum,* essential oil, chemical composition, antibacterial activity, gas chromatography-mass spectrometry (GC-MS).

## INTRODUCTION

*Z. bungeanum* Maxim, commonly named huajiao in China, belongs to the Rutaceae family and has been widely used as a food spice in Chinese cuisine for its unique taste known as "ma" (a pungent taste). The fruits of *Z. bungeanum* contain many phytochemicals, such as essential oil, amides, alkaloids, coumarin, flavonoids and phenols (Xiong et al., 1995; Wu et al., 2004). In China, most of *Z. bungeanum* are sold in the form of dry fruits or powders, and some are processed into flavored oil or sauce. *Z. bungeanum* is also applied as a popular folk medicine for the treatment of pathogenic wind, dampness, itch, abdominal pain, eczema, vomiting and diarrhea (Li, 1982). In addition, it was reported that *Z. bungeanum* was effective for inflammatory diseases due to its inhibitory activity on NO production (Tezuka et al., 2001). Beside its pharmacological function, *Z. bungeanum* was also reported to inhibit fungi (Gong et al., 2009) and deter oviposition as well as larval feeding (Ge and Weston, 1995).

Microbial contaminations in foods not only result in food quality and shelf life reduction, but also lead to various human infections. The Gram-positive bacterium *S. aureus* and Gram-negative bacterium *E. coli* are two main food spoilage bacteria, which cause food-borne illness. *S. aureus* has been recognized as the main cause of post-operative wounds infections and food poisoning (Mylotte et al., 1987), while *E. coli* has been known as the cause of diarrhea (Friedman et al., 2002). Many synthetic preservatives have been utilized to control food-borne pathogens and food spoilage bacteria, such as sodium benzoate, sorbic acid and potassium sorbate. However, these synthetic chemicals are sometimes associated with adverse effects including hypersensitivity, allergic reaction, and immunity suppression (Cakir et al., 2005). Moreover, these preservatives always cause the

*Corresponding author. E-mail: zhaozhifeng_scu@126.com. Tel.: +86 28 85405236. Fax: +86 28 85405137.

development of drug-resistant bacteria after long-term usage. Therefore, much effort has been made to search for natural preservatives, including the extracts or essential oils isolated from various species of edible and medicinal plants, herbs and spices (Longaray Delamare et al., 2007; Ozkan et al., 2010; Singh et al., 2005).

Essential oils are aromatic and volatile oily liquids obtained from plant materials as secondary metabolites. They are normally found in leaves, stems, barks and fruits (Oussalah et al., 2006). Many essential oils are classified as "generally regarded as safe" (GRAS) by the United State Food and Drug Administration (FDA). Previous studies have showed that plant essential oils possess the capacity to inhibit microorganisms and extend the shelf life of vegetables (Du et al., 2009; Ponce et al., 2004). Consequently, essential oils are considered as one of the most promising natural preservatives in food industry among different plant products due to their significant antimicrobial activity and safety.

To the best of our knowledge, there is no information linked to the influence of *Z*. *bungeanum* essential oil on the growth and morphological structure of food-borne bacteria. Therefore, in the present study, the main objectives were to identify the chemical components of the essential oil from the fruits of *Z*. *bungeanum* by gas chromatography-mass spectrometry (GC-MS), to evaluate its antibacterial activity against some common food spoilage bacteria, to investigate its effect on the growth of bacteria in meat-based model media, and to determine its effect on the morphological structure of *B. subtilis* by transmission electron microscope (TEM).

## MATERIALS AND METHODS

### Plant materials and chemicals

The dried fruits of *Z*. *bungeanum* were provided by Sichuan Wufeng Lihong Co., Ltd. (Hanyuan, Ya'an, China). These specimens were harvested by hand in August, 2009. The moisture of the dried samples was about 11%. A voucher specimen was preserved at the Key Laboratory of Food Science and Technology of Sichuan Province, Sichuan University, China.

Agar, beef extract, and peptone were purchased from Bio-Ketai (Langsan Ketai Biological Products Co., Ltd., China). Potassium sorbate was obtained from Sichuan Changwei Pharmaceutical Co., Ltd., China. Phosphate balanced solution (PBS), glutaraldehyde, osmium tetroxide, acetone and epoxy were purchased from Sigma-Aldrich (St. Louis, MO). The solvents for GC-MS analysis were of high performance liquid chromatography (HPLC)-grade. All other reagents used were of analytical grade.

### Preparation of the essential oil

The plant materials were crushed into powders (about 40 granularities) with a mixer (JYL-350, Jiuyang Co., Ltd., China). To prevent a loss of volatile compounds, the powders were prepared just before use. Twenty grams of the powders were subjected to hydro-distillation for 2 h by using a modified Clevenger-type apparatus with 200 ml of deionized water. The essential oil floating on the top of the condensed water was obtained in a yield of 5.6% (w/w) after drying with anhydrous $Na_2SO_4$. The oil was stored in the dark at 4°C until used.

### GC-MS analysis

The analysis of the essential oil was performed by GC-MS (Trace DSQ II, Thermo Fisher Corporation, USA). The column used was a TR-5 capillary column (30 m × 0.25 mm internal diameter, 0.25 m film thickness). The oven temperature was held at 60°C for 4 min, then programmed to 240 at 4°C /min. Helium (99.99%) was the carrier gas at a flow rate of 1.0 ml/min. Injector, MS transfer line, and ion source temperatures were set at 250, 280 and 200°C, respectively. Mass scanning was from 40 to 500 amu. An aliquot (1.0 µl) of the diluted samples (1/100 in *n*-hexane, w/v) were injected manually.

Compounds were identified by comparing their GC retention times and mass spectra with those of Adams (2001), NIST (3.0) mass spectra library and relative references (Zhao et al., 2004 a, b). The percentage composition of the essential oil was computed by the normalization method from the GC peak areas.

### The tested bacteria

Five common food-borne bacteria were used in this study, including four Gram-positive bacteria: *S. aureus* ATCC25923, *B. subtilis* ATCC21216, *B. cereus* ATCC10231 and *B. laterosporus laubach* ATCC 64, and one Gram-negative bacterium: *E. coli* ATCC25922. All the strains were maintained on medium at 4°C and were sub-cultured every month in our laboratory. Before used, the strains will be cultured and examined by morphological and physiological characteristics experiment, to ensure the strains never has any mutation. Besides, the strains sub-cultured for three times will not be used again.

### *In vitro* antibacterial assay

#### *Well diffusion method*

The antibacterial activity of the essential oil was determined by agar well diffusion method (Oke et al., 2009). The inoculums of bacteria were prepared from overnight cultures, and suspensions were adjusted to $10^7$ CFU/ml with deionized water. Petri dishes with 20 mL of culture medium were prepared, previously inoculated with 200 µl of the bacterial suspension. The wells with a diameter of 6 mm, were injected with 20 µl of the essential oil dissolved in 50% ethanol (9 mg/well). Potassium sorbate (0.5%, pH 4.5) was used as the positive control. After staying at 4°C for 2 h to allow dispersal, these plates were incubated at 37°C for 24 h. The antibacterial activity was evaluated by measuring the zone of inhibition against the test bacteria. The measurements were done basically from the edge of the zone to the edge of the well.

### Determination of minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The MIC and MBC values of the essential oil were measured by the broth dilution method (Zeng et al., 2011). Different concentrations of the essential oil (0.16-40 mg/ml) were added to the sterilized test tubes, which containing 2 ml of nutrient broth. The test bacteria suspension was added to each tube to keep the final inoculum size of $10^7$ CFU/ml. The tubes were incubated at 37°C for 24 h and were evaluated by macroscopic evaluation. The lowest concentration that inhibited the bacterial growth was taken as the MIC. All the MIC samples were then streaked on nutrient agar plates and incubated at 37°C for 24 h. The concentration at which show no bacteria

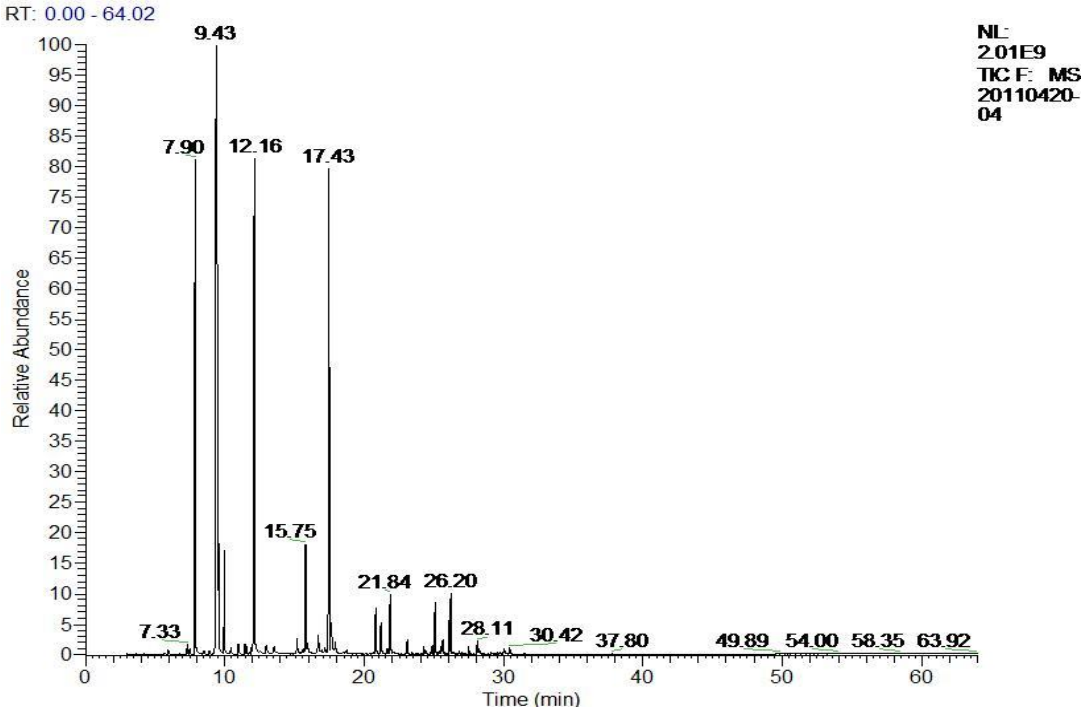


**Figure 1.** Total ion chromatogram of essential oil from *Zanthoxylum bungeanum*. The samples injected into the GC-MS system were a 1% solution of the essential oil.

growth was determined as MBC.

#### Effect of the essential oil on viable counts of bacteria using food model media

To investigate the effect of the essential oil on viable counts of bacteria in meat-based model media, experiments were performed with autoclaved beef extract (BE, 3% in deionized water). BE media were adjusted to pH 7.2 (Gutierrez et al., 2008). For viable counts, tubes containing 10 ml of BE media and a bacterial suspension (approximately $10^7$ CFU/ml) of *S. aureus*, *B. subtilis* and *E. coli* were inoculated with MIC of the essential oil, and kept at 37°C. Samples for viable counts were taken out at 0, 30, 60, 90, 120, and 150 min time intervals. The viable plate counts were monitored as the follows: 0.1 mL of each treatment culture was diluted into 0.9 mL deionized water, to generate a dilution series of the samples ($10^{-1}$-$10^{-6}$ times), and 0.1 ml of each diluted sample was spread on the surface of nutrient agar. The colonies were counted after 24 h of incubation at 37°C. The controls were inoculated without essential oil for each bacterial strain with the same experimental condition described earlier.

#### Observation with TEM

TEM observation for *B. subtilis* was determined by our previous method (Zeng et al., 2011). Nutrient broth containing *B. subtilis* (cultured for 24 h) was centrifugal at 5000 rpm at 25°C for 10 min, and the precipitate was washed three times with PBS (0.1 M, pH 7.4). Glutaraldehyde (0.5%) was added to the precipitate and kept for 15 min at 4°C. *B. subtilis* cells were collected by 20 min centrifugation (15000 rpm, 4°C). The cells were fixed with 3% glutaraldehyde for 2 h, post-fixed with 1% osmium tetroxide for 3 h, dehydrated with graded acetone solutions at 4°C for 20 min and embedded in epoxy. Thin sections cutted by a microtome (Ultracut-E, Reichert-Jung, Austria) were stained with 1% aqueous toluidine blue at 40°C, then observed by TEM (H-600IV, Hitachi, Japan).

#### Statistical analysis

All analysis of the data was performed using SPSS 13.0 software (SPSS Inc., 223 South Wacker Drive, Chicago, USA). A probability value at $P < 0.05$ was considered statistically significant. All data were expressed as means ± standard deviation of triplicate measurements.

## RESULTS AND DISCUSSION

### Chemical composition of the essential oil

The essential oil from the fruits of *Z*. *bungeanum* isolated by hydro-distillation method presented a light yellow color. Result of the GC-MS analysis showed that *Z*. *bungeanum* essential oil contained mostly volatile compounds (Figure 1), with the majority appearing in the first 30 min. The identified chemical components together with their retention times and percentage composition are given in Table 1. A total of 28 compounds were identified from the oil, which represented 94.30% of the total extraction. The oil was dominant by oxygenated (41.65%) and non-oxygenated hydrocarbons (52.65%). The major compounds were limonene (29.36%), linalool (17.02%),

**Table 1.** Chemical identity and relative composition of the essential oil from the fruits of *Zanthoxylum bungeanum.*

| No | RT (min) | Compound | Composition (%) |
|---|---|---|---|
| 1 | 5.96 | α-Pinene | 0.11 |
| 2 | 7.33 | α-Phellandrene | 0.24 |
| 3 | 7.51 | (1S)-6,6-Dimethyl-2-methylene-bicyclo[3.1.1]heptanes | 0.13 |
| 4 | 7.90 | β-Pinene | 14.31 |
| 5 | 9.42 | Limonene | 29.36 |
| 6 | 9.55 | Eucalyptol | 1.71 |
| 7 | 9.62 | (E)-3,7-Dimethyl-1,3,6-octatriene | 1.48 |
| 8 | 9.99 | (Z)-3,7-Dimethyl-1,3,6-octatriene | 2.2 |
| 9 | 10.45 | γ-Terpinene | 0.14 |
| 10 | 10.97 | α-Methyl-α-[4-methyl-3-pentenyl]oxiranemethanol | 0.27 |
| 11 | 11.44 | Terpinolen | 0.25 |
| 12 | 12.16 | Linalool | 17.02 |
| 13 | 13.00 | Trans-p-mentha-2,8-dienol | 0.31 |
| 14 | 15.14 | 4-Carvomenthenol | 0.48 |
| 15 | 15.75 | α-Terpinol | 2.65 |
| 16 | 16.64 | (Z)-Carveol | 0.58 |
| 17 | 17.43 | Linalyl acetate | 13.84 |
| 18 | 17.57 | D-Carvone | 0.93 |
| 19 | 17.88 | Phenethyl acetate | 0.26 |
| 20 | 20.81 | Terpinyl acetate | 1.14 |
| 21 | 21.18 | Neryl acetate | 0.74 |
| 22 | 21.84 | Geranyl acetate | 1.40 |
| 23 | 23.08 | Caryophyllene | 0.40 |
| 24 | 24.26 | α-Caryophyllene | 0.18 |
| 25 | 25.07 | β-Cubebene | 1.26 |
| 26 | 25.61 | α-Muurolene | 1.11 |
| 27 | 26.20 | δ-Cadinene | 1.48 |
| 28 | 28.11 | Espatuleno | 0.32 |
| Total | | | 94.30% |

RT, Retention time.

β-pinene (14.31%) and linalyl acetate (13.84%). The result was a little different from a previous study, in which the main components were shown to be linalyl acetate, following by linalool, limonene, 4-terpinenol, 1, 8-cineole and myrcene (Yang, 2008). Another report showed that eucalyptol, linalool, 3-carene, acetosyringone and 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol were the major components of *Z*. *bungeanum* oil obtained by an improved solvent-free microwave (Wang et al., 2006). This reason can be explained that the chemical composition of *Z*. *bungeanum* essential oil varied with the genetic characteristics, grown conditions and extract method as the previous report (Chen and He, 2009).

## Antibacterial activity

The essential oil showed a variable degree of antibacterial activity against the different strains tested. As shown in Table 2, the Gram-positive bacterium, *S. aureus* was the most sensitive to the oil, while the Gram-negative bacterium, *E. coli*, was the most resistant. Special attention should be paid to the inhibitory zones of *S. aureus*, the oil exhibited stronger inhibitory activity (20.0±0.5 mm) against *S. aureus* than potassium sorbate (13.0±0.4) used commonly in food industry currently. In addition, MIC and MBC values for each assay of test bacteria showed a significant correlation with the inhibition zones of the essential oil ($P < 0.05$). The highest inhibitory activity was against *S. aureus* which showed the lowest MIC (1.25 mg/ml) and MBC (2.50 mg/ml). The negative control of 50% ethanol did not show any inhibition effect on the growth of test bacteria. The results of the present study are similar to previous reports, in which *S. aureus* showed more sensitivity to the essential oils than did *E. coli* (Matasyoh et al., 2009; Nanasombat and Wimuttigosol, 2011). The resistance of Gram-negative bacteria can be attributed to their outer

**Table 2.** Antibacterial activity of essential oil from the fruits of *Zanthoxylum bungeanum.*

| Bacterial strains | Inhibition zone diameter (mm) | | Essential oil (mg/ml) | |
|---|---|---|---|---|
| | Essential oil | Positive control | MIC | MBC |
| *E. coli* | 5.4±0.1[5)] | 13.3±0.3 | 5.0 | 20.0 |
| *S. aureus* | 20.0±0.5 | 13.0±0.4 | 1.25 | 2.5 |
| *B. subtilis* | 8.9±0.3 | 12.9±0.6 | 2.5 | 10.0 |
| *B. laubach* | 10.3±0.1 | 13.2±0.5 | 1.25 | 5.0 |
| *B. cereus* | 7.9±0.2 | 12.8±0.3 | 2.5 | 5.0 |

The concentration of essential oil was 9 mg/well; Potassium sorbate (0.5%, pH 4.5) was used as positive control; Each value is expressed as mean ± SD (n = 3); MIC, Minimum inhibitory concentrations; MBC, Minimum bactericidal concentrations.

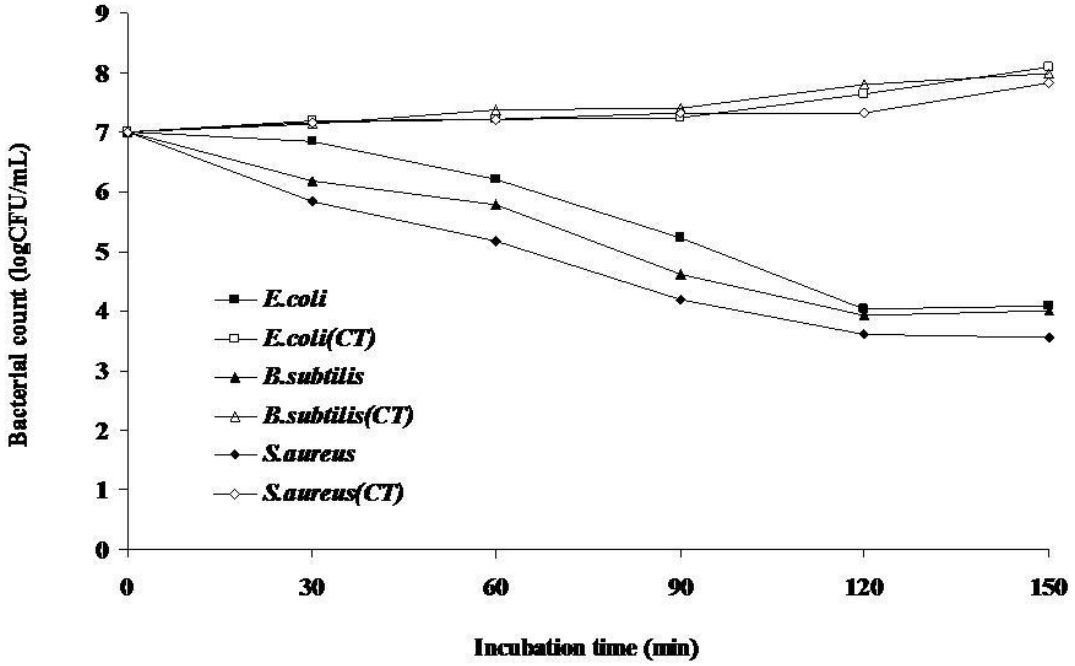


**Figure 2.** Effect of the essential oil from the fruits of *Zanthoxylum bungeanum* on viability of the tested bacteria in beef extracts media. The concentration of the essential oil was set at MIC concentration for the tested bacteria. CT: control without treatment.

membrane which is rich in lipopolysaccharide molecules presenting a barrier to the penetration of many antimicrobial components in the essential oils. Additionally, the resistance is also associated with the enzymes in the periplasmic space, which can break down the antimicrobial components of essential oils (Sivarooban et al., 2008). The antibacterial activity of *Z. bungeanum* essential oil could be associated with the main constituents such as limonene, linalool, β-pinene, and α-terpineol, which are known to possess significant antibacterial activity (Van Vuuren and Viljoen, 2007; Kim et al., 1995; Stojkovic et al., 2008).

The effects of essential oil on viable counts of *S. aureus*, *B. subtilis* and *E. coli* in BE media were shown in Figure 2. The bacteria without essential oil grew slowly in the first 90 min, and then obvious growth was observed in the last 60 min. Conversely, bacteria exposed to essential oil showed a steep decline in CFU numbers during the first 120 min, and then the bacteria count remained stable until the end of assay. The exposure time of the essential oil for complete inhibition of cell viability of all the tested bacteria was found to be 120 min, when the essential oil exerted its maximum bactericidal activity at MIC level. Similarly, *S. aureus* was found to be the most sensitive, since the oil exerted maximum bacterial activity against *S. aureus* with the lowest concentration. Therefore, the MIC of the oil had a severe effect on the cell viability of the tested bacteria.

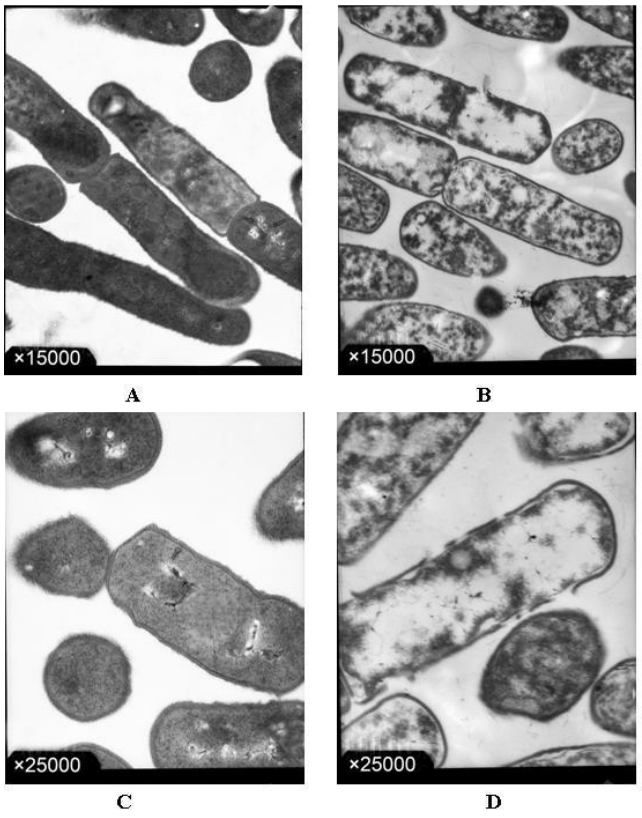


**Figure 3.** Transmission electron microscope diagram of *B. subtilis* in the absence (A, C) and presence (B, D) of the essential oil from *Zanthoxylum bungeanum*.

TEM photomicrographs of *B. subtilis* in the presence or absence of the essential oil were shown in Figure 3. *B. subtilis* cells in control group showed normal morphology (claviform, elliptical), regular outlined cell walls, cytoplasmic membranes lying closely to the cell walls, cytoplasm regularly filled the entire cells (Figures 3A and C). After treatment with the essential oil at MIC level, the cells were seriously damaged (Figure 3B) compared with the cells in the control. Cells with incomplete cytoplasm could be seen distributed throughout field of view. In Figure 3D (at greater magnification), the cell walls and membranes of the *B. subtilis* can be seen to be partially disintegrated, allowing the outflow of electron-translucent cytoplasm. The localized separation of cell membranes from cell walls could also be observed from the photomicrographs. The essential oil showed obvious influence on the morphological structure of *B. subtilis*. Previous findings suggested that essential oils can alter cell fluidity/permeability and functions by entering between the fatty acyl chains making up membrane lipid bilayers and disrupt the lipid packing (Hammer et al., 2004; Sikkema et al., 1995). The loss of differential permeability character of the cytoplasmic membrane is frequently identified as the cause of cell death.

In conclusion, the present study confirmed the chemical composition and antibacterial activity of the essential oil from the fruits of *Z. bungeanum*. The results suggested that the oil of *Z. bungeanum* could be used as antibacterial agent in foods. Further studies will focus on the cytotoxicity of the essential oil.

## ACKNOWLEDGEMENT

Authors are thankful to Sichuan Wufeng Lihong Food Co., Ltd. for their financial and material support (Contract Number: 08H0018).

### REFERENCES

Adams RP (2001). Identification of essential oil components by gas chromatography/quadrupolemass spectroscopy. Allured Publishing Corporation, Carol Stream, IL, USA.

Cakir A, Kordali S, Kilic H, Kaya E (2005). Antifungal properties of essential oil and crude extracts of *Hypericum linarioides* Bosse. Biochem. Syst. Ecol., 33: 245-256.

Chen X, He RS (2009). Analysis and comparison on volatile oil of *Zanthoxylum planispinum* var. *dintanensis* and *Z. bungeanum* Dahongpao in Maoxian county of Sichuan province by GC-MS. J. Anhui Agri. Sci., 37: 1879-1880.

Du WX, Olsen CW, Avena-Bustillos RJ, Mchugh TH, Levin CE, Mandrell R, Friedman M (2009). Antibacterial effects of Allspice, Garlic, and Oregano essential oils in tomato films determined by overlay and vapor-phase methods. J. Food Sci., 74: M390-M397.

Friedman M, Henika PR, Mandrell RE (2002). Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenese*, and *Salmonella enterica*. J. Food Protect., 65: 1545-1560.

Ge XS, Weston PA (1995). Ovipositional and feeding deterrent from Chinese prickly ash against angoumois grain moth (lepidoptera, gelechiidae). J. Econ. Entomol., 88: 1771-1775.

Gong YW, Huang YF, Zhou LG, Shi XY, Guo ZJ, Wang MG, Jiang WB (2009). Chemical composition and antifungal activity of the fruit oil of *Z. bungeanum* Maxim. (Rutaceae) from China. J. Essent. Oil Res., 21: 174-178.

Gutierrez J, Barry-Ryan C, Bourke P (2008). The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. Int. J. Food Microbiol., 124: 91-97.

Hammer KA, Carson CF, Riley TV (2004). Antifungal effects of *Melaleuca alternifolia* (tea tree) oil and its components on *Candida albicans*, *Candida glabrata* and *Saccharomyces cerevisiae*. J. Antimicrob. Chemother., 53: 1081-1085.

Kim J, Marshall MR, Wei CI (1995). Antibacterial activity of some essential oil components against five foodborne pathogens. J. Agric. Food Chem., 43: 2839-2845.

Li SZ (1982). Compendium of Materia Medica. People's Med. Publishing House, Beijing, China. p. 202.

Longaray Delamare AP, Moschen-Pistorello IT, Artico L, Atti-Serafini L, Echeverrigaray S (2007). Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. Food Chem., 100: 603-608.

Matasyoh JC, Maiyo ZC, Ngure RM (2009), Chepkorir R. Chemical composition and antimicrobial activity of the essential oil of *Coriandrum sativum*. Food Chem., 113: 526-529.

Mylotte JM, McDermott C, Spooner JA (1987). Prospective study of 114 consecutive episodes of *Staphylococcus aureus* bacteria. Rev. Infect. Dis., 9: 891-907.

Nanasombat S, Wimuttigosol P (2011). Antimicrobial and Antioxidant Activity of Spice Essential Oils. Food Sci. Biotechnol., 20: 45-53.

Oke F, Aslim B, Ozturk S, Altundag S (2009). Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten. Food Chem., 112: 874-879.

Oussalah M, Caillet S, Saucier L, Lacroix M (2006). Inhibitory effects of

selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157:H7, *Salmonella* Typhimurium, *Staphylococcus aureus* and *Listeria monocytogenes*. Food Control, 18: 414-420.

Ozkan G, Sagdic O, Gokturk RS, Unal O, Albayrak S (2010). Study on chemical composition and biological activities of essential oil and extract from *Salvia pisidica*. LWT-Food Sci. Technol., 43: 186-190.

Ponce AG, del Valle C, Roura SI (2004). Shelf life of leafy vegetables treated with natural essential oils. J. Food Sci., 69: FMS50-FMS56.

Sikkema J, de Bont JAM, Poolman B (1995). Mechanisms of membrane toxicity of hydrocarbons. Microb. Rev., 59: 201-222.

Singh G, Marimuthu P, De Heluani CS, Catalan C (2005). Antimicrobial and Antioxidant Potentials of Essential Oil and Acetone Extract of *Myristica fragrans* Houtt. (Aril Part). J. Food Sci., 70: M141-M148.

Sivarooban T, Hettiarachchy NS, Johnson MG (2008). Physical and antimicrobial properties of grape seed extract, nisin, and EDTA incorporated soy protein edible films. Food Res. Int., 41: 781-785.

Stojkovic D, Sokovic M, Glamoclija J, Dzamic A, Ristic M, Fahal A, Khalid S, Djuic I, Petrovic S (2008). Susceptibility of three clinical isolates of *actinomodura madurae* to α- Pinene, the bioactive agent of *pinus pinaster* turpentine oil. Arch. Biol. Sci., 64: 697-701.

Tezuka Y, Irikawa S, Kaneko T, Banskota AH, Nagaoka T, Xiong QB, Hase K, Kadota S (2001). Screening of Chinese herbal drug extracts for inhibitory activity on nitric oxide production and identification of an active compound of *Zanthoxylum bungeanum*. J. Ethnopharmacol., 77: 209-27.

Van Vuuren SF, Viljoen AM (2007). Antimicrobial activity of limonene enantiomers and 1,8-cineole alone and in combination. Flavour Frag. J., 22: 540-544.

Wang ZM, Ding L, Li TC, Zhou X, Wang L, Zhang HQ, Liu L, Li Y, Liu ZH, Wang HJ, Zeng H, He H (2006). Improved solvent-free microwave extraction of essential oil from dried *Cuminum cyminum* L. and *Z. bungeanum* Maxim. J. Chromatogr. A, 1102: 11-17.

Wu SR, Kan JQ, Liu CF (2004). Research on the bioactivity constiuents and application of *Zanthoxylum bungeanum*. China Food Addit. 2: 75-78.

Xiong QB, Shi DW, Mizuo M (1995). Flavonol glucosides in pericarps of *Zanthoxylum bungeanum*. Phytochem., 39: 723-725.

Yang XG (2008). Aroma Constituents and Alkylamides of Red and Green Huajiao (*Z. bungeanum* and *Zanthoxylum schinifolium*). J. Agric. Food Chem., 56: 1689-1696.

Zeng WC, Zhu RX, Jia LR, Gao H, Zheng Y, Sun Q (2011). Chemical composition, antimicrobial and antioxidant activities of essential oil from *Gnaphlium affine*. Food Chem. Toxicol., 49: 1322-1328.

Zhao ZF, Lei M, Lei SR, Ou Yang HX, Yan ZN. (2004a). Experiment study on the extraction of *Xanthoxylin* in different solvent [J]. China Food Addit., 4: 18-21.

Zhao ZF, Lei M, Lei SR, Ou Yang HX, Yan ZN. (2004b). Analysis of chemical component of volatile oil from two kinds of *Zanthoxylum bungeanum* maxin in Sichuan [J]. China Food Addit., 10: 39-42.