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Antibacterial activity of Java turmeric (*Curcuma xanthorrhiza* Roxb.) extract against *Klebsiella pneumoniae* isolated from several vegetables

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Abstract

Klebsiella pneumoniae is a foodborne pathogen associated with pneumoniae. Multiresistance to antibiotics of *K. pneumoniae* is a significant public health treat. Recently, the use of natural products such as herbs to inhibit the growth of pathogens is increasing. Java turmeric (*Curcuma xanthorrhiza* Roxb.) has been reported to possess antibacterial activity against foodborne pathogens. Unfortunately, the antibacterial activity of java turmeric extract against the resistance to multiantibiotics of *K. pneumoniae* has not been investigated. In this study, the antibacterial activity of Java turmeric extract was tested against 24 isolates of resistant *K. pneumoniae* that was isolated from several vegetables; lettuce, cucumber, tomato and carrot, using the methods recommended by the Clinical and Laboratory Standard Institute (CSLI), including disc diffusion method, minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC) and killing time at concentration 0× MIC, ½× MIC, 1× MIC, 2× MIC and 4× MIC with predetermined time of 0, 0.25 , 0.5, 1, 2 and 4 h. The results showed that Java turmeric extract is susceptible to all resistant *K. pneumoniae* with inhibition zones ranging from 8.67 ± 0.58 to 10.00 ± 0.00 mm. The MIC and MBC values for the *K. pneumoniae* isolates against all bacterial isolates was 1.25 and 2.5 mg/ml, respectively. The killing time curve shows the reduction of resistant *K. pneumoniae* cells is fast acting; > 3 log10 within less than 15 min at 4× MIC (5.0 mg/ml). Finally, the isolates were completely killed at 4× MIC for 15 min. In conclusion, the Java turmeric extracts can be developed as natural antimicrobial agent to inhibit the growth of *K. pneumoniae* in food system.

Introduction

Klebsiella species is one of the members of enterobacteriaceae, Gram-negative, facultative anaerobic, non motile and non-flagellated bacilli, which are less well known bacteria that have also been implicated in foodborne diseases. *Klebsiella pneumoniae* is ubiquitous in environment such as vegetation, soil and surface water. Furthermore, the isolates from the environment were found to be as virulent as the clinical strains (Bagley, 1985; Struve and Krogfelt, 2004; Balestrino *et al*., 2008). Although these bacteria may often be associated to food spoilage, it also may occasionally cause what is often described as opportunistic pathogens. It has been considered as opportunistic pathogens because adults and healthy persons are not at high risk to develop infections and illness but young children, the elderly and immunocompromised as well as people who practice poor sanitation and inadequate nutrition are at high risk. *Klebsiella* spp. are ubiquitous in the nature and have two widespread habitats; either being in the environment such as water, sewage and soil otherwise being the mucosal surfaces of mammals such as humans or horses (Podschun and Ullmann, 1998). Outbreaks of gastrointestinal diseases from fresh produce were also found to be triggered by bacterial contamination especially associated to the Enterobacteriaceae family (Falomir *et al.*, 2010). In Houston, Texas, *K. pneumoniae* infection caused the patient to suffer from the symptoms of gastroenteritis which led to multiorgan failure. It was also reported that dried bush okra *(Corchorus olitorius)*, African spider herbs *(Cleome gynandra)* both in Botswana and fruit juices from Libya showed the presence of *K. pneumoniae* (Mpuchane and Gashe, 1996; Sabota *et al*., 1998; Ghengesh *et al*., 2005; Puspanadan *et al*., 2012). Fresh sprout products in Canada was found to be contaminated with *K. pneumoniae* (Lawley *et al*., 2012). For that reason, edible medicinal plants can be used as one of the alternative methods to minimize or eliminate the foodborne pathogens since they had

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been found to be rich in antimicrobial agents.

The findings of the new antimicrobial compounds in natural products especially from plant origins became one of the remarkable alternatives for treatments since they are rich in numerous varieties of secondary metabolites such as alkaloids, flavanoids, tannins, antharaquinones and phenolic compounds with antimicrobial properties (Olajuyigbe and Afolayan, 2012). In the period before 2007, approximately half of all the drugs recorded worldwide are from natural products or their synthetic derivatives (Fernandes Júnior *et al*., 2014).

One of the large genus of rhizomatous herbs is *Curcuma* (Zingiberaceae) which can be found in tropical and subtropical regions especially in Indonesia, India, Thailand, the Malay Archipelago, Indochina as well as Northern Australia. The rhizomes of *Curcuma* have been generally used in the form of powder as flavours in native dishes and constituent in many traditional medicine to treat various ailments (Jantan *et al*., 2012). Our interest mainly is on the *Curcuma xanthorrhiza* Roxburgh which is also known as *temulawak* in Malaysia. The bioactive compound in *C. xanthorrhiza*, xanthorrhizol (Figure 1) exhibited anticariogenic activity against *Streptococcus mutans* (Rukayadi and Hwang, 2006), antifungal against *C. albicans, C. glabrata, C. guilliermondii, C. krusei, C. parapsilosis* and *C. tropicalis* (Rukayadi *et al*., 2006), anti-Malassezia activity against *M. furfur* and *M. pachydermatis* (Rukayadi and Hwang, 2007a) and antimycotic against opportunistic filamentous fungi (Rukayadi and Hwang, 2007b). As the results, this study is aimed to investigate the antimicrobial activity of *C. xanthorrhiza* Roxb. extract against *K. pneumoniae* that had been isolated from vegetables.

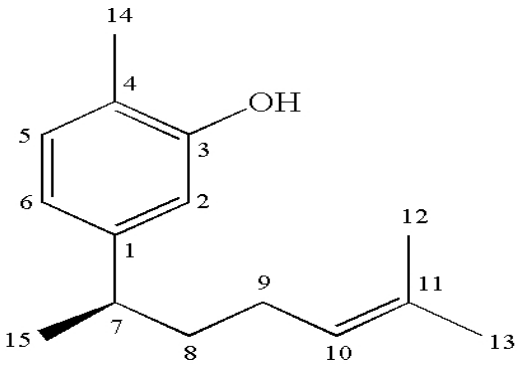

Figure 1. Structure of xanthorrhizol (Rukayadi *et al*., 2009)

Materials and Methods

Plant material and preparation of methanolic extract

The rhizome of *temulawak* or *Curcuma xanthorrhiza* Roxb. were obtained from Laboratory of Natural Product, Institute of Bioscience (IBS), Universiti Putra Malaysia, Serdang. The dried rhizome of *C. xanthorrhiza* was grounded into powder and extracted using methanol (Rukayadi *et al*., 2008). 100 g of powdered dried medicinal plant was soaked in 400 ml of absolute methanol for 48 hours at room temperature and repeated twice. The extract was then filtered using Whatman filter paper No. 2 (150 mm) (Sigma-Aldrich, St. Louis, USA) and further treatment with rotary vacuum evaporator to yield concentrated methanol extract at 50˚C at Laboratory of Biochemistry, Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang. The methanolic extract was then preserved at 4˚C for further use (Soniya *et al*., 2013). For the antimicrobial assay, the 0.1 g of extract was dissolved in 1 ml dimethylsufoxide (DMSO) to obtain stock solution. Throughout this study, 1% or 10 mg/ml of the plant extract was used. A 10% DMSO was used as a negative control since it does not kill the tested bacteria in this study.

Klebsiella *strains and inoculum preparation*

The 24 isolates of *K. pneumoniae* were obtained from Bacteriology Food Safety Laboratory, Universiti Putra Malaysia, Serdang. The isolates were isolated from raw vegetables (Puspanadan *et al*., 2012). *K. pneumoniae* ATCC 13773 from the American Type Culture Collection (Rockville, MD, USA) was used as a reference control throughout the study. All the test strains were sub-cultured on Mueller Hinton Agar medium (MHA), incubated at 37˚C for 24 h and stored at 4˚C in the refrigerator to maintain stock culture.

Disc-diffusion method

The susceptibility screening of the bacterial isolates to the plant extract and chlorhexidine (CHX) as a control was determined using the Kirby-Bauer diffusion method (Bauer *et al*., 1966) to conduct initial determination of antimicrobial activity of the crude methanol extract of *C. xanthorrhiza.* Sterile filter paper was used to prepare 6 mm in diameter discs and 10 µl of 1% plant extract was impregnated on the disc. A 1% chlorhexidine and 10% DMSO was included in the test and served as positive and negative control respectively. All of the plates were then incubated at 37˚C for 24 hours. The determination was done in duplicates and performed 3 times for each of the isolates. After 24 hours of incubation, the plates were observed for any clear zone and the diameters for each isolates were measured in millimeters. A clear inhibition zone around impregnated disc was the

Table 1. Summary of inhibition zone, minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) for *K. pneumoniae* isolates

Strains		Inhibition zone (mm ± SD)	MIC (mg/ml)	MBC (mg/ml)
ATCC 13773		12.33 ± 0.58	0.078	5.0
Lettuce (L)	L1	8.67 ± 0.58	1.25	2.5
	L2	9.68 ± 0.58	1.25	2.5
	L3	9.33 ± 0.58	1.25	2.5
	L4	8.67 ± 0.58	1.25	2.5
	L5	9.33 ± 0.58	1.25	2.5
	L6	9.33 ± 0.58	1.25	2.5
Tomato (T)	T1	10.00 ± 0.00	1.25	2.5
	T2	9.67 ± 0.58	1.25	2.5
	T3	9.67 ± 0.58	1.25	2.5
	T4	8.67 ± 0.58	1.25	2.5
	T5	9.33 ± 0.58	1.25	2.5
	T6	9.33 ± 0.58	1.25	2.5
Carrot (C)	C1	9.33 ± 0.58	1.25	2.5
	C2	9.67 ± 0.58	1.25	2.5
	C3	9.00 ± 0.00	1.25	2.5
	C4	10.00 ± 0.00	1.25	2.5
	C5	9.33 ± 0.58	1.25	2.5
	C6	8.67 ± 0.58	1.25	2.5
Cucumber (K)	K1	9.67 ± 0.58	1.25	2.5
	K2	9.67 ± 0.58	1.25	2.5
	K3	10.00 ± 0.00	1.25	2.5
	K4	9.67 ± 0.58	1.25	2.5
	K5	9.33 ± 0.58	1.25	2.5
	K6	9.33 ± 0.58	1.25	2.5

indication of the presence of antibacterial activity.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *C. xanthorrhiza* for each isolates was performed on Mueller Hinton agar and broth according to the method described in the guidelines of Clinical Laboratory Standard Institute M7-A9 (2012) with modification. In brief, this test was done in a 96-well round bottom microtitre plate for all of the *K. pneumoniae* isolates using broth microdilution methods; the bacterial suspension was made from an overnight culture and diluted to a turbidity comparable to that of a 0.5 McFarland standard and further diluted with broth so that the final concentration of the bacteria was approximately 10^6 cfu/ml. The 96-well microtitre was filled with a two-fold dilution of *C. xanthorrhiza* extract stock solution mixed with the bacterial isolates in Mueller Hinton broth (MHB). The purpose of the two-fold dilution is to reduce the concentration of the solution by a factor of two which then reduces the original concentration by one half. The dilution on the wells was started from the column at position 12 of the microtiter plates. The microdilution method was done by adding 100 µl of inoculums from the MHB medium into each of the microwells except for the column at position 1 that served as the negative control (fresh medium without inoculum or antibacterial agent). Then, 100 µl of plant extract was added into the column at position 12 on the microwell. The extract was then mixed using the pipette and 100 µl of the mixture was subsequently transferred into the next microwell at column position 11. The procedure was repeated and it went from right to left on the microtitre plate. After all dilution was performed, 100 µl of the suspension at column position 3 was discarded and column 2 served as the positive control (fresh medium and inoculum). The microtiter plate was then incubated at 37˚C for 24 hours aerobically. The MIC is defined as the lowest concentration of an antimicrobial agent that prevents visible growth of test microorganism in the 96-well microtiter plates. The first well with no visible growth after the incubation period was taken as the MIC.

The minimum bactericidal concentration (MBC) is the minimal concentration of antimicrobial that kills the inoculums and can be determined from broth dilution MIC test by subculturing into MHA medium (approximately of 10 µl) without any antimicrobial agents (French, 2006; Rukayadi *et al.*, 2009). The positive controls (fresh medium and inoculums) which were in column 2 and wells in column 1 as the negative controls (antimicrobial agent free wells) were also included in the MBC test. The plate was then incubated at 37˚C for 24 hours aerobically. MBC is defined as the lowest concentration of antimicrobial agent whereby the *K. pneumoniae* in the culture were killed or the lowest concentration with no visible growth on MHA plates.

Table 2. Summary of time-kill

Strains		Time-kill Summary
ATCC 13773		Killed completely after 0.25, 0.5 and 2 hour with 8× MIC, 4× MIC and 2× MIC, respectively
Lettuce	L1	Killed completely after 0.25 hour with 4× MIC
	L2	Killed completely after 0.25 hour with 4× MIC
	L3	Killed completely after 0.25 hour with 4× MIC
	L4	Killed completely after 0.25 hour with 4× MIC
	L5	Killed completely after 0.25 hour with 4× MIC
	L6	Killed completely after 0.25 hour with 4× MIC
Tomato	T1	Killed completely after 0.25 hour with 4× MIC
	T2	Killed completely after 0.25 hour with 4× MIC
	T3	Killed completely after 0.25 hour with 4× MIC
	T4	Killed completely after 0.25 hour with 4× MIC
	T5	Killed completely after 0.25 hour with 4× MIC
	T6	Killed completely after 0.25 hour with 4× MIC
Carrot	C1	Killed completely after 0.25 hour with 4× MIC
	C2	Killed completely after 0.25 hour with 4× MIC
	C3	Killed completely after 0.25 hour with 4× MIC
	C4	Killed completely after 0.25 hour with 4× MIC
	C5	Killed completely after 0.25 hour with 4× MIC
	C6	Killed completely after 0.25 hour with 4× MIC
Cucumber	K1	Killed completely after 0.25 and 4 hour with 4× MIC and 2× MIC, respectively
	K2	Killed completely after 0.25 and 4 hour with 4× MIC and 2× MIC, respectively
	K3	Killed completely after 0.25 and 4 hour with 4× MIC and 2× MIC, respectively
	K4	Killed completely after 0.25 and 4 hour with 4× MIC and 2× MIC, respectively
	K5	Killed completely after 0.25 and 4 hour with 4× MIC and 2× MIC, respectively
	K6	Killed completely after 0.25 and 4 hour with 4× MIC and 2× MIC, respectively

Time-kill assay

Time-kill assay was used to examine the rate at which concentration of an antimicrobial agent kills a bacterial isolate and also to evaluate new antimicrobial agent. Time-kill assay was performed in MHB medium according to the method of Lorian (2005) and Pankey and Ashcraft (2009), with some modifications. There were four different concentrations of *C. xanthorrhiza* extract used to kill the resistant *K. pneumoniae* isolates and as a reference strain (ATCC 13773): 0× MIC, ½× MIC, 1× MIC, 2× MIC and 4× MIC. All isolates were incubated at 37 ˚C with 150 rpm agitation at pre-determined time points (0, 15 and 30 minutes as well 1, 2 and 4 hours). At each of the time point, 100 µl aliquots were removed from each concentration and spread on the MHA medium and incubated at 37˚C for 24 hours. The number of colonies that was visible on the plates was counted and recorded as log (cfu/ml).

Results and Discussion

Medicinal plants are being actively exploited to expose their natural antimicrobial properties. There are many researches on the antimicrobial activity of *C. xanthorrhiza* reported but not yet exposed to *K. pneumoniae*. The solvent used to extract *C. xanthorrhiza* was methanol and plant extract in organic solvent, methanol showed more consistent extraction in terms of antimicrobial substances from medicinal plants compared to those extracted in ethanol, water or hexane (Parekh *et al*., 2005; Hafidh *et al.*, 2011; Soniya *et al.*, 2013). The effects of *C. xanthorrhiza* extracts against twenty four isolates of *K. pneumoniae* isolated from several vegetables were identified and the results of the inhibition zone were summarized in Table 1. The inhibition zone of the extract at 10 mg/mL showed that *K. pneumonaie* ATCC 13773 exhibited 12.33 ± 0.58 mm and the isolates from tomato(T1), cucumber (C4), lettuce (L2) and carrot (K3) was 10.00 ± 0.00 mm, 10.00 ± 0.00 mm, 9.68 ± 0.58 mm and 10.00 ± 0.00 mm respectively. From the results of inhibition zone, *C. xanthorrhiza* extract is effective against *K. pneumoniae* ATCC 13773 and from those strains isolated from Malaysian vegetables. Puspanadan *et al.* (2013) reported that all twenty four isolates of *K. pneumoniae* isolated from Malaysian vegetables are resistant to aminoglycosides, penicillin, tetracyclines and sulfonamides. From previous researches done on *K. pneumoniae*, a number of medicinal plants

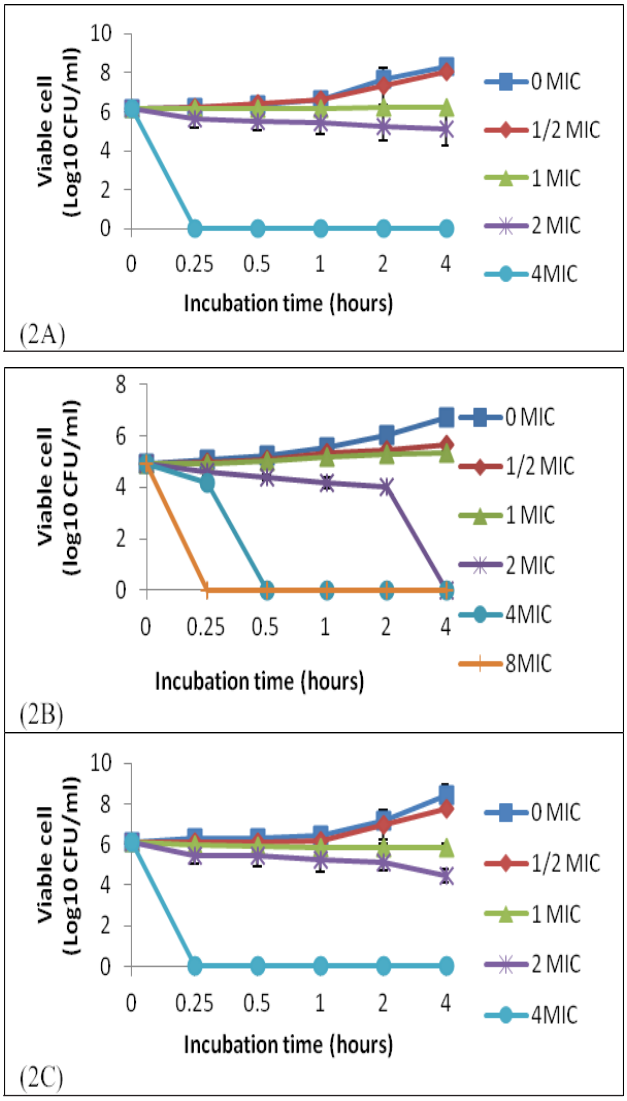

Figure 2. Representative time-kill curve of *K. pneumoniae* isolates from several vegetables; 2A and 2B (0, 0.625, 1.25, 2.5 and 5.0 mg/ml) at concentration of 0× MIC, 1/2× MIC, 1× MIC, 2× MIC and 4× MIC including *K. pneumoniae* ATCC 13773; 2C (0, 0.078, 0.156, 0.312 and 0.624 mg/ml) at concentration of 0× MIC, 1/2× MIC, 1× MIC, 2× MIC, 4× MIC and 8× MIC

extracts cannot inhibit the growth of these bacteria (Okigbo and Omodamiro, 2007; Joshi *et al.*, 2011). Generally, medicinal plant extracts were effective against Gram-positive compared to Gram-negative bacteria. These could be explained by the distinctive feature of Gram-negative bacteria that have a double membrane surrounding the bacteria which make the outer membrane excludes certain drug, antibiotics and antibacterial agents from penetrating the cell therefore making Gram-negative bacteria the most resistant to plant extract in contrast of Gram-positive bacteria (Reynolds, 1996; Zaidan *et al.*, 2005; Gangoué-Piéboji *et al.*, 2009; Hafidh *et al.*, 2011). This might be due to the fact that Gram-negative bacteria have the hydrophilic cell wall structure consisted of lipo-polysaccharide (LPS) that acts as a defence system to block the penetration of hydrophobic components and avoids the accumulation of plant extract in target cell membrane. On the other hand, this outer membrane has the capability to pass the lipophilic compounds and macromolecules. Permeating outer membrane of the microorganisms is the prerequisite condition for any solute to have antibacterial activity (Bezic *et al.*, 2003; Bajpai *et al.*, 2007; Kang *et al.*, 2011).

For this study, the *K. pneumoniae* isolates which were resistant to aminoglycosides, penicillin, tetracyclines and sulfonamides (Puspanadan *et al.*, 2012) were selected to identify which concentration of the plants extracts that can inhibit the bacterial growth. Quantitative analyses of the *C. xanthorrhiza* on the antimicrobial activity were obtained through the determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values. From table 1, the MIC and MBC for all *K. pneumoniae* isolates were 1.25 and 5.0 mg/ml respectively while for ATCC 13773 the MIC and MBC was 0.078 and 5.0 mg/ml respectively.

In the time-kill assay, the results was presented in terms of the changes in the $\log_{10}$ cfu/ml of viable colonies which indicated that the extract exhibited a significant bactericidal activity. According to Scheetz *et al.* (2007) and Olajuyigbe and Afolayan (2012), once the viable colony counts was equal to 3 $\log_{10}$ cfu/ml or have greater reduction was deliberated to have bactericidal activity. The results of the time-kill assay were summarized in Table 2 and Figure 2 is the representative of the time-kill curve graph for the isolates. For strains that were isolated from lettuce (L), tomato (T) and carrot (C), plant extract can inhibit completely after 0.25 hours of incubation time at concentration of 4× MIC while the isolate from cucumber (K) the plant extract can kill completely after 0.25 and 0.5 hours of incubation time at concentration of 4× MIC and 2× MIC, respectively. For the *K. pneumoniae* ATCC 13773, the plant extract can kill the strain at concentration of 8× MIC, 4× MIC and 2× MIC for 0.25, 0.5 and 2 hours respectively.

Conclusion

From the findings, it showed that *C. xanthorrhiza* extracts exhibits the potential natural antibacterial candidate as a food sanitizer for vegetables. The results obtained can be further studied aiming to understand the antibacterial mechanisms of the compound found in the *C. xanthorrhiza* so that the benefits of the plants can be exploited.

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References

Bagley, S. T. 1985. Habitat association of *Klebsiella* species. Infection Control: 52-58.

Bajpai, V. K., Rahman, A., Choi, U. K., Youn, S. J. and Kang, S. C. 2007. Inhibitory parameters of the essential oil and various extracts of *Metasequoia glyptostroboides* Miki ex Hu to reduce food spoilage and food-borne pathogens. Food Chemistry 105(3): 1061-1066.

Balestrino, D., Ghigo, J. M., Charbonnel, N., Haagensen, J. A. and Forestier, C. 2008. The characterization of functions involved in the establishment and maturation of *Klebsiella pneumoniae* in vitro biofilm reveals dual roles for surface exopolysaccharides. Environmental Microbiology 10(3): 685-701.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C. T. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology 45(4): 493-496

Bezić, N., Skočibušić, M., Dunkić, V. and Radonić, A. 2003. Composition and antimicrobial activity of *Achillea clavennae* L. essential oil. Phytotherapy Research 17(9): 1037-1040.

Clinical and Laboratory Standard Institute (CLSI). 2012. Reference methods for Dilution Antimicrobial Susceptibility Test for Bacteria That Grow Aerobically. Approved Standard – Ninth Edition M07-A9. Wyane, PA, USA.

Falomir, M. P., Gozalbo, D. and Rico, H. 2010. Coliform bacteria in fresh vegetables: from cultivated lands to consumers. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology 2: 1175-1181.

Fernandes Júnior, A., da Silva, G. S., Barbosa, L. N., Alves, F. C. B., Andrade, B. F. M. T., Albano, M. and di Stasi, L. C. 2014. Medicinal plants from the Brazilian savanna with antibacterial properties. European Journal of Medicinal Plants 4(1): 1-13.

French, G. L. 2006. Bactericidal agents in the treatment of MRSA infections—the potential role of daptomycin. Journal of Antimicrobial Chemotherapy 58(6): 1107-1117.

Gangoue-Pieboji, J., Eze, N., Djintchui, A. N., Ngameni, B., Tsabang, N., Pegnyemb, D. E., Biyiti, P., Ngassam, P., Koulla-Shiro, S. and Galleni, M. 2009. The *in-vitro* antimicrobial activity of some medicinal plants against β-lactam- resistant bacteria. The Journal of Infection in Developing Countries 3(09): 671-680.

Ghenghesh, K. S., Belhaj, K., El-Amin, W. B., El-Nefathi, S. E. and Zalmum, A. 2005. Microbiological quality of fruit juices sold in Tripoli–Libya. Food Control 16(10): 855-858.

Hafidh, R. R., Abdulamir, A. S., Vern, L. S., Bakar, F. A., Abas, F., Jahanshiri, F. and Sekawi, Z. 2011. Inhibition of growth of highly resistant bacterial and fungal pathogens by a natural product. The Open Microbiology Journal 5: 96-106.

Jantan, I., Saputri, F. C., Qaisar, M. N. and Buang, F. 2012. Correlation between chemical composition of Curcuma domestica and *Curcuma xanthorrhiza* and their antioxidant effect on human low-density lipoprotein oxidation. Evidence-Based Complementary and Alternative Medicine ID438356:1-10.

Joshi, B., Sah, G. P., Basnet, B. B., Bhatt, M. R., Sharma, D., Subedi, K., Pandey, J. and Malla, R. 2011. Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem). Journal of Microbiology and Antimicrobials 3(1): 1-7.

Kang, C. G., Hah, D. S., Kim, C. H., Kim, Y. H., Kim, E. and Kim, J. S. 2011. Evaluation of antimicrobial activity of the methanol extracts from 8 traditional medicinal plants. Toxicological Research 27(1): 31-36.

Lawley, R., Curtis, L. and Davis, J. 2012. The Food Safety Hazard Guidebook. 2nd edition. United Kingdon, Cambrige: The Royal Society of Chemistry.

Lorian, V. 2005. Antibiotics in Laboratory Medicine. 5th edition. USA, Lippincott: Williams and Wilkins.

Mpuchane, S. F. and Gashe, B. A. 1996. Presence of *Escherichia coli, Klebsiella pneumoniae* and *Enterobacter* species in dried bush okra *(Corchorus olitorius)* and African spider herb *(Cleome gynandra)*. Food Control 7(3): 169-172.

Okigbo, R. N. and Omodamiro, O. D. 2007. Antimicrobial effect of leaf extracts of pigeon pea (*Cajanus cajan* (L.) Millsp.) on some human pathogens. Journal of Herbs, Spices and Medicinal Plants 12(1-2): 117-127.

Olajuyigbe, O. O. and Afolayan, A. J. 2012. In vitro antibacterial and time-kill evaluation of the *Erythrina caffra* Thunb. extract against bacteria associated with diarrhoea. The Scientific World Journal ID738314.

Olajuyigbe, O. O. and Afolayan, A. J. 2012. *In vitro* pharmacological activity of the crude acetone extract of *Erythrina caffra* Thunb: antibacterial and antifungal assessment. Journal of Medicinal Plants Research 6(9): 1713-1720.

Pankey, G. A. and Ashcraft, D. S. 2009. *In vitro* antibacterial activity of tigecycline against resistant Gram-negative bacilli and enterococci by time–kill assay. Diagnostic Microbiology and Infectious Disease 64(3): 300-304.

Parekh, J., Jadeja, D. and Chanda, S. 2005. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. Turkish Journal of Biology 29: 203-210.

Podschun, R. and Ullmann, U. 1998. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clinical Microbiology Reviews 11(4): 589-603.

Puspanadan, S., Afsah-Hejri, L., John, Y. H. T., Rukayadi,

Y., Loo, Y. Y., Nillian, E., Kuan, C. H., Goh, S. G., Chang, W. S., Lye, Y. L., Mohd Shahril, N., Yoshitsugu, N., Nishibuchi, M. and Son, R. 2013. Characterization of extended-spectrum β-lactamases (ESBLs) producers in *Klebsiella pneumoniae* by genotypic and phenotypic method. International Food Research Journal 20(3): 1479-1483.

Puspanadan, S., Afsah-Hejri, L., Loo, Y. Y., Nillian, E., Kuan, C. H., Goh, S. G., Chang, W. S., Lye, Y. L., John, Y. H. T., Rukayadi, Y., Yoshitsugu, N., Nishibuchi, M. and Son, R. 2012. Detection of *Klebsiella pneumoniae* in raw vegetables using Most Probable Number-Polymerase Chain Reaction (MPN-PCR).International Food Research Journal 19(4): 1757-1762.

Reynolds, J. E. F. 1996. Martindale the extra harmacopoeia. 31st edition. London: Royal Pharmaceutical Society of Great Britain.

Rukayadi, Y. and Hwang, J. K. 2006. *In vitro* activity of xanthorrhizol against Streptococcus mutans biofilms. Letters in Applied Microbiology 42(4): 400-404.

Rukayadi, Y., Yong, D. and Hwang, J. K. 2006. *In vitro* anticandidal activity of xanthorrhizol isolated from *Curcuma xanthorrhiza* Roxb. Journal of Antimicrobial Chemotherapy 57(6): 1231-1234.

Rukayadi, Y. and Hwang, J. K. 2007a. *In vitro* anti-Malassezia activity of xanthorrhizol isolated from *Curcuma xanthorrhiza* Roxb. Letters in Applied Microbiology 44(2): 126-130.

Rukayadi, Y. and Hwang, J. K. 2007b. *In vitro* antimycotic activity of xanthorrhizol isolated from *Curcuma xanthorrhiza* Roxb. against opportunistic filamentous fungi. Phytotherapy Research 21(5): 434-438.

Rukayadi, Y., Shim, J. S. and Hwang, J. K. 2008. Screening of Thai medicinal plants for anticandidal activity. Mycoses 51(4): 308-312.

Rukayadi, Y., Lee, K., Han, S., Yong, D. and Hwang, J. K. 2009. *In vitro* activities of panduratin A against clinical *Staphylococcus* strains. Antimicrobial Agents and Chemotherapy 53(10): 4529-4532.

Sabota, J. M., Hoppes, W. L., Ziegler, J. R., DuPont, H., Mathewson, J. and Rutecki, G. W. 1998. A new variant of food poisoning: enteroinvasive *Klebsiella pneumoniae* and *Escherichia coli* sepsis from a contaminated hamburger. The American Journal of Gastroenterology 93(1): 118-119.

Scheetz, M. H., Qi, C., Warren, J. R., Postelnick, M. J., Zembower, T., Obias, A. and Noskin, G. A. 2007. *In vitro* activities of various antimicrobials alone and in combination with tigecycline against carbapenem -intermediate or -resistant *Acinetobacter baumannii.* Antimicrobial Agents and Chemotherapy 51(5): 1621-1626.

Soniya, M., Kuberan, T., Anitha, S. and Sankareswari, P. 2013. *In vitro* antibacterial activity of plant extracts against Grampositive and Gramnegative pathogenic bacteria. International Journal of Microbiological Immunology 2: 1-5

Struve, C. and Krogfelt, K. A. 2004. Pathogenic potential of environmental *Klebsiella pneumoniae* isolates. Environmental Microbiology 6(6): 584-590.

Zaidan, M. R., Noor Rain, A., Badrul, A. R., Adlin, A., Norazah, A. and Zakiah, I. 2005. *In vitro* screening of five local medicinal plants for antibacterial activity using disc diffusion method. Tropical Biomedicine 22(2): 165-170.