Asian Pacific Journal of Tropical Biomedicine

Original Article

doi: www.apjtb.org

Antibacterial activity and bioactive compounds of 50% hydroethanolic extract of *Alpinia zerumbet* (Pers.) B.L. Burtt & R.M. Sm.

Ratree Tavichakorntrakool[1,2], Aroonlug Lulitanond[1,2], Arunnee Sangka[1,2], Seksit Sungkeeree[1,2], Natthida Weerapreeyakul[3]✉

[1]*Centre for Research and Development of Medical Diagnostic Laboratories, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand*

[2]*Department of Clinical Microbiology, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand*

[3]*Division of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand*

ARTICLE INFO

Article history:
Received 1 March 2019
Revision 26 March 2019
Accepted 29 April 2019
Available online 24 May 2019

Keywords:
Antibacterial activity
Phenolic
Flavonoid
Alpinia zerumbet
HPLC

ABSTRACT

Objective: To evaluate antibacterial activity and the bioactive compounds of 50% hydroethanolic extract of *Alpinia zerumbet* (*A. zerumbet*) rhizomes.

Methods: Eight reference microbial strains including two Gram-positive bacteria [*Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 29212)] and six Gram-negative bacteria [*Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATTC 700603), *Proteus mirabilis* (DMST 8212), *Salmonella enterica* subsp. *enterica* serovar Vellore. (ATCC 15611), *Shigella flexneri* (ATCC 12022) and *Pseudomonas aeruginosa* (ATCC 27853)], were used to test antimicrobial susceptibility by the broth microdilution method. Bioactive compounds were analyzed by using HPLC.

Results: The minimum inhibitory concentration values of *A. zerumbet* extract were 8 mg/mL for *Staphylococcus aureus*, *Escherichia coli* and *Shigella flexneri* and 16 mg/mL for *Enterococcus faecalis* and the other four Gram-negative bacilli. HPLC chromatograms revealed that the *A. zerumbet* extract contained hydroxybenzoic acids, hydroxycinnamic acids and flavonoids.

Conclusions: The constituents of *A. zerumbet* rhizomes could be a potential source of antibacterial compounds, warranting further study of *A. zerumbet* extract.

1. Introduction

The antibacterial resistance in pathogenic bacteria has significantly increased for the treatment. Currently, the natural products from plant extracts have been used as antibacterial agents. *Alpinia zerumbet* (Pers.) B.L. Burtt & R.M. Sm. (*A. zerumbet*) syn. *Catimbium speciosum* (J.C.Wendl.) Holttum.–widely grown in tropical and subtropical zones–has been exploited in traditional medicine as well as cuisine[1–3]. *A. zerumbet* or shell ginger is in *Alpinia* group and is the largest genus in the family Zingiberaceae[1,4]. In Thailand, it is commonly known as Kha-Kom. Many studies reported that *A. zerumbet* possesses high potential bioactivity such as anticancer[5], antioxidant[6], antiinflammatory, analgesic, antiallergic, neuroprotective effects[4], anti-diabetes[2], anti-obesity

✉Corresponding author: Natthida Weerapreeyakul, Division of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand.
Tel.: +66 43 202 378
Fax: +66 43 202 379
E-mail: natthida@kku.ac.th

Funding: This work was supported by Khon Kaen University through the Plant Genetic Conservation Project Under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn (Project no. 591414 and 600626).

How to cite this article: Tavichakorntrakool R, Lulitanond A, Sangka A, Sungkeeree S, Weerapreeyakul N. Antibacterial activity and bioactive compounds of 50% hydroethanolic extract of *Alpinia zerumbet* (Pers.) B.L. Burtt & R.M. Sm. Asian Pac J Trop Biomed 2019; 9(5): 204-208.

and antiatherogenic effects[2,7], and specifically antimicrobial activity[8]. According to these numerous health benefits, *A. zerumbet* may promote longevity[2].

The major compounds in *A. zerumbet* rhizomes, stems and fresh leaves are kavalactones (*i.e.*, dihydro-5,6-dehydrokawain, and 5,6-dehydrokawain), with other minor constituents including volatile oils, phenols, and fatty acids[2]. The other plant that contains dihydro-5,6-dehydrokawain is *Piper methysticum*[9]. This compound is found in two different plant families (Zingiberaceae and Piperaceae), which have long been consumed with no toxicity to human, therefore, it is enough to warrant for possible clinical applications. However, the detected amount of those phytochemicals was varied in different parts of *A. zerumbet* depending on growing and harvesting season, and agricultural area. Hence, the extraction is a pivotal way to obtain high content of phytochemicals. The solvent used for extraction should be concerned to avoid the risk of toxicity besides good agricultural practice. With a long history of using *A. zerumbet*, the bioactive compounds of *A. zerumbet* according to Thai traditional folklore have not yet been completely determined. We, therefore, evaluated the antibacterial activity and the bioactive compounds of *A. zerumbet* extract with 50% water and ethanol.

2. Materials and methods

2.1. Bacteria

The reference microbial strains included (1) two Gram-positive bacteria [*Staphylococcus aureus* (*S. aureus*) ATCC 29213 and *Enterococcus faecalis* (*E. faecalis*) ATCC 29212], and (2) six Gram-negative bacteria [*Proteus mirabilis* (*P. mirabilis*) DMST 8212, *Escherichia coli* (*E. coli*) ATCC 25922, *Klebsiella pneumoniae* (*K. pneumoniae*) ATTC 700603, *Salmonella enterica* (*S. enterica*) subsp. *enterica* serovar Vellore. ATCC 15611, *Shigella flexneri* (*S. flexneri*) ATCC 12022 and *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 27853]. The Gram-positive bacteria were grown on blood agar (Oxoid Ltd., Basingstoke, England) while the Gram-negative bacteria were grown on MacConkey agar (Oxoid Ltd., Basingstoke, England), respectively prior to testing.

2.2. Plant collection

A. zerumbet was collected from Chaiyaphum province under the Plant Genetic Conservation Project conducted with the permission of the Royal Initiative of her Royal Highness Princess Maha Chakri Sirindhorn, and The Bureau of the Royal Household and Electricity Generating Authority of Thailand. *A. zerumbet* was authenticated using the herbarium collection (TT-OC-SK-857) at the Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen province, Thailand by Assistant Professor Thaweesak Thitimetharoch.

2.3. Extraction of A. zerumbet

A. zerumbet rhizomes were ground and macerated with 50% hydro-ethanolic (1 kg per 6 liters) for 7 d at room temperature (25-28 ℃)[5]. Filtration was conducted to get filtrate which was evaporated by a rotary evaporator with 40 ℃ in water bath temperature. The water content was removed by using freeze-dryer to obtain the dry crude extracts. The crude extract was stored in a desiccator protected from light at room temperature until used.

2.4. Antibacterial activity by broth microdilution method

The experiment was conducted according to the standard method[10]. Eight bacterial suspensions were made up to 0.5 McFarland and diluted to 1:100. *A. zerumbet* extract (0.125-32 mg/mL) and gentamicin (0.125-32 μg/mL) (Oxoid, Basingstoke, England) were prepared in the 96-well plates at 37 ℃ for 24 h[11].

The lowest concentration of *A. zerumbet* extract or gentamicin that could inhibit the visible growth of bacteria was expressed as the minimum inhibitory concentration (MIC). Furthermore, the lowest concentration of *A. zerumbet* extract or gentamicin without growth on agar plate at 37 ℃, for 24 h was expressed as the minimum bactericidal concentration (MBC).

2.5. Phytochemical identification by using HPLC

The presence of phenolics and flavonoids in the *A. zerumbet* extracts was identified and quantified using HPLC (LC-20AC, Shimadzu, Japan) as per previous method[12] with minor modification. The reference standards (purchased from Sigma-Aldrich, USA) were gallic acid, vanillic acid, *p*-hydroxybenzoic acid, protocatechuic acid, syringic acid, chlorogenic acid, *p*-coumaric acid, caffeic acid, apigenin, kaempferol, ferulic acid, sinapic acid, myricetin, rutin, and quercetin. The *A. zerumbet* extract was prepared in DMSO and filtered through a membrane filter (0.45 μm). The injection volume was 20 μL with flow rate of 0.8 mL/min. The reverse phase column was an Inetsil® ODS-3 C18 column (4.6 mm × 250 mm, 5 μm; Hichrom Limited, Berks, U.K.). The gradient mobile phase was a mixture of 1% acetic acid in DI water (solvent X) and acetonitrile (solvent Y), which was eluted at 38 ℃ following the previous study[12] with some modifications. The gradient elution started from 5%-9% solvent Y (0-5 min); 9% solvent Y (5-15 min); 9%-11% solvent Y (15-22 min); 11%-18% solvent Y (22-38 min); 18%-23% solvent Y (38-43 min); 23%-90% solvent Y (43-44 min); 90%-80% solvent Y (44-45 min); isocratic at 80% solvent Y (45-55 min); 80 isocratic -5% solvent Y (55-60 min), and 5% solvent Y was used between individual runs for 5 min for equilibration. The detection wavelength of the UV-diode array detector (SPD-M20A, Shimadzu, Japan) was set for phenolic and flavonoids compounds at 280 nm and 370 nm, respectively. The retention times and their peak area of standard phenolic compounds were determined. The contents of phenolics and flavonoids in the extracts were calculated from the linear regression equations obtained from standard curves, which were plotted between the peak area (y axis) against concentrations of standard compounds (x axis) ranged from 6.25-100 μg/mL with the correlation coefficients ($R^2 > 0.99$). Data were expressed as mean ± standard deviation.

3. Results

3.1. MIC and MBC of extract of A. zerumbet against eight microbial strains

The antibacterial activities of 50% hydro-ethanolic extract of *A. zerumbet* are shown in Table 1. The ranges of MICs and MBCs of

Table 1. Antibacterial activities of *A. zerumbet* extract *versus* gentamicin by broth microdilution method.

Bacterial strains	*A. zerumbet* extract (mg/mL)		Gentamicin (μg/mL)	
	MIC	MBC	MIC	MBC
Gram-positive bacteria				
E. faecalis ATCC 29212	16.0	32.0	16.0	16.0
S. aureus ATCC 29213	8.0	8.0	0.5	1.0
Gram-negative bacteria				
E. coli ATCC 25922	8.0	16.0	1.0	1.0
K. pneumoniae ATTC 700603	16.0	16.0	16.0	16.0
P. mirabilis DMST 8212	16.0	16.0	16.0	16.0
P. aeruginosa ATCC 27853	16.0	16.0	1.0	2.0
S. enterica subsp. *enterica* serovar Vellore. ATCC 15611	16.0	16.0	2.0	2.0
S. flexneri ATCC 12022	8.0	16.0	2.0	2.0

MIC = Minimum inhibitory concentration; MBC = Minimum bactericidal concentration.

A. zerumbet extract were 8-32 mg/mL. The MIC values of the *A. zerumbet* extract were 8 mg/mL for *S. aureus*, *E. coli* and *S. flexneri*, and 16 mg/mL for *E. faecalis*, and the other four Gram-negative bacilli. The MBC values of *A. zerumbet* extract were 8 and 32 mg/mL for *S. aureus* and *E. faecalis*, respectively. The MBC values of the *A. zerumbet* extract were 16 mg/mL for all Gram-negative bacilli.

3.2. Phytochemical components of extract of A. zerumbet by HPLC

The phytochemical components of *A. zerumbet* extract were analyzed by HPLC. The HPLC chromatograms of *A. zerumbet* extract are illustrated in Figure 1B and 2B in comparison with the HPLC chromatograms of phenolic (Figure 1A) and flavonoid standards (Figure 2A). The contents of phenolic acid and flavonoid compounds of *A. zerumbet* extract were calculated from the peak area (Table 2). *A. zerumbet* extract was found to contain hydroxybenzoic acids (*i.e.*, gallic acid, syringic acid, protocatechuic acid and *p*-hydroxybenzoic acid), hydroxycinnamic acids (*i.e.*, ferulic acid) and flavonoids (*i.e.*, quercetin, myricetin, rutin and kaempferol). Flavonoids were predominantly found in *A. zerumbet* according to high detected amount.

Table 2. The phenolic and flavonoid contents in the extract of *A. zerumbet* based on HPLC analysis.

Compounds (Peak number)	Detected amount (μg/g)
Hydroxybenzoic acids	
Gallic acid (1)	158.29 ± 1.45
Protocatechuic acid (2)	110.64 ± 2.15
p-Hydroxybenzoic acid (3)	85.45 ± 0.02
Vanillic acid (5)	ND
Syringic acid (7)	139.32 ± 2.89
Total	493.70 ± 3.73
Hydroxycinnamic acids	
Chlorogenic acid (4)	ND
Caffeic acid (6)	ND
p-Coumaric acid (8)	ND
Ferulic acid (9)	85.62 ± 0.52
Sinapic acid (10)	ND
Total	85.62 ± 0.52
Flavonoids	
Rutin (1)	656.47 ± 2.63
Myricetin (2)	25 067.45 ± 4.96
Quercetin (3)	9 068.47 ± 6.40
Apigenin (4)	ND
Kaempferol (5)	518.61 ± 0.45
Total	35 310.99 ± 4.93

ND = Not detected.

4. Discussion

Some plants are used as traditional medicines for treatment of the urinary tract[11] and gastrointestinal tract infectious diseases. *A. zerumbet* is a traditional medicine used as an antibacterial agent[3]. The crude extract of *A. zerumbet* was used because the antibacterial action may need the combination or synergistic effects of several bioactive compounds in the extract. Our results showed that the bioactive compounds of *A. zerumbet* extract were the flavonoids and phenolic compounds, which were previously reported to have antibacterial activity[13–17]. The flavonoids found in this study were myricetin, quercetin, rutin and kaempferol, respectively. In addition, the most common types of phenolic compounds were hydroxybenzoic acids group (including gallic acid, syringic acid, protocatechuic acid and *p*-hydroxybenzoic acid) and hydroxycinnamic acid group (ferulic acid). These compounds in the *A. zerumbet* extract had some potential of the antibacterial activity.

Notably, dihydro-5,6-dehydrokavain and 5,6-dehydrokawain are the major compounds found in *A. zerumbet* in the amount of 80–410 mg/g of fresh weight, and ≤100 mg/g, respectively[18]. Other compounds include (i) its derivative hispidin, converted by acid hydrolysis by gastric juice and subsequently metabolized by hepatic microsomal CYP2C9[19,20], (ii) phenolics, (iii) flavonoids and (iv) volatile oils (≤150 mg/g) such as rutin, catechins, kaempferol, flavanones, labdadienes, zerumins, camphor and methyl cinnamate, 1,8-cineol, isothymol, thymol, eugenol, chalcone cardamonin and alpinetin[1,21,22]. The essential oils found in *A. zerumbet* exerted antifungal effect on plant fungal pathogen[1]. According to the reported high amount of dihydro-5,6-dehydrokavain in *A. zerumbet* or minor amount of oils, it cannot be ruled out in our study that the polar solvent extraction cannot yield these bioactive compounds.

Both Gram-positive bacteria (*S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212) and Gram-negative bacteria (*E. coli* ATCC 25922, *K. pneumoniae* ATTC 700603, *P. mirabilis* DMST 8212, *S. enterica* subsp. *enterica* serovar Vellore. ATCC 15611, *S. flexneri* ATCC 12022 and *P. aeruginosa* ATCC 27853) were selected to study because they were the reference strains that related with the urinary tract and gastrointestinal tract infectious diseases. Our data found that the *A. zerumbet* extract could inhibit both two Gram-positive and six Gram-negative reference bacteria in difference concentrations. *S. aureus* ATCC 29213 was the most sensitive strain to *A. zerumbet* extract. These findings corresponded to the previous study in which the *A. zerumbet* extract exerted inhibitory effect on *S. aureus*[13]. This previous study showed that the flower extract obtained from

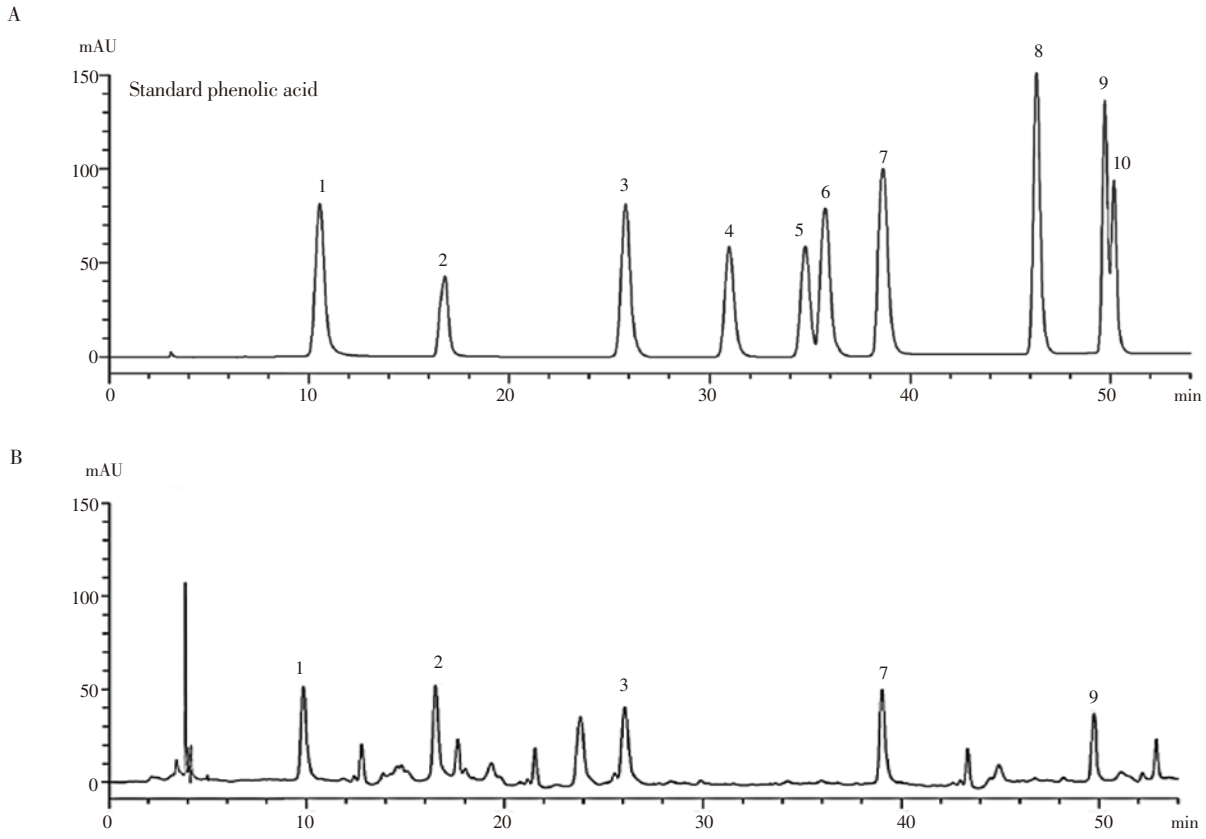

Figure 1. HPLC chromatograms of (A) standard phenolic acids including (1) gallic acid, (2) protocatechuic acid, (3) *p*-hydroxybenzoic acid, (4) chlorogenic acid, (5) vanillic acid, (6) caffeic acid, (7) syringic acid, (8) *p*-coumaric acid, (9) ferulic acid and (10) sinapic acid and (B) HPLC chromatograms of *A. zerumbet* extract.

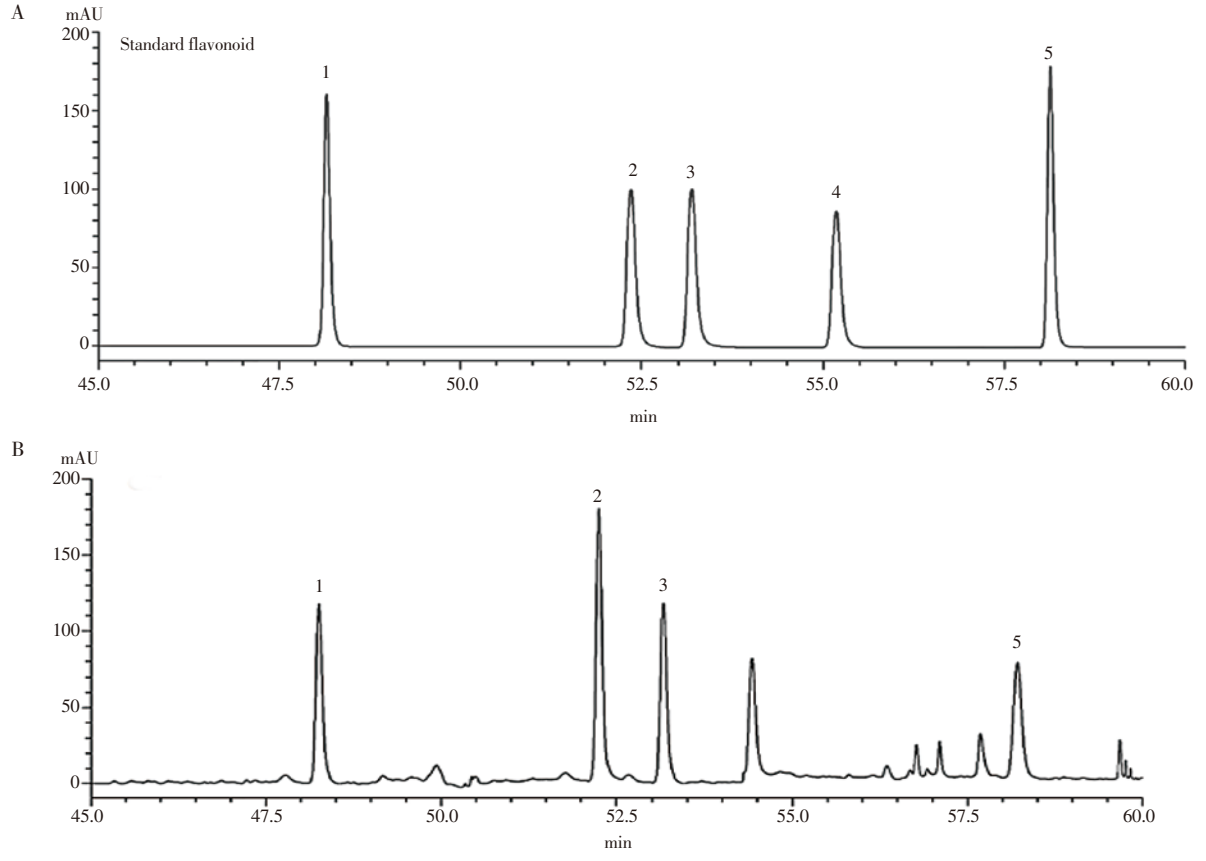

Figure 2. HPLC chromatograms of (A) standard flavonoids including (1) rutin, (2) myricetin, (3) quercetin, (4) apigenin and (5) kaempferol and (B) HPLC chromatograms of *A. zerumbet* extract.

methanol, hexane, dichloromethane and ethyl acetate had a greater extent of inhibition on *S. aureus* but showed no activity against *P. aeruginosa*[13] when compared to the extract of *A. zerumbet* rhizome in our study. *A. zerumbet* has been long historically used with no side effect but more benefits[2,18]. These evidences confirm that the extract of *A. zerumbet* rhizome exhibits the antibacterial activity and may be potential for developing product for treatment of urinary tract infection.

In conclusion, *A. zerumbet* extract had antibacterial effect on two Gram-positive and six Gram-negative reference bacteria especially *S. aureus* ATCC 29213. However, the antibacterial activities against the clinical bacterial strains should be further evaluated in the future. Moreover, the total phenolics and flavonoids contents in the extract of *A. zerumbet* rhizome were 85 and ~35,000 μg/g, respectively. Thus it is necessary to conduct the solvent extraction to achieve high yield of bioactive compounds of *A. zerumbet* extract for further clinical use.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Funding

This work was supported by Khon Kaen University through the Plant Genetic Conservation Project Under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn (Project no. 591414 and 600626).

References

[1] Tawata S, Fukuta M, Xuan TD, Deba F. Total utilization of tropical plants *Leucaena leucocephala* and *Alpinia zerumbet*. *J Pestic Sci* 2008; **33**(1): 40-43.

[2] Teschke R, Xuan TD. Viewpoint: A contributory role of shell ginger (*Alpinia zerumbet* (Pers.) B.L. Burtt & R.M. Sm) for human longevity in Okinawa, Japan? *Nutrients* 2018; **10**(2): E166.

[3] Victório CP. Therapeutic value of the genus *Alpinia*, Zingiberaceae. *Rev Bras Farmacogn* 2011; **21**(1): 194-201.

[4] Ghosh S, Rangan L. *Alpinia*: The gold mine of future therapeutics. *3 Biotech* 2013; **3**(3): 173-185.

[5] Machana S, Weerapreeyakul N, Barusrux S, Nonpunya A, Sripanidkulchai B, Thitimetharoch T. Cytotoxic and apoptotic effects of six herbal plants against the human hepatocarcinoma (HepG2) cell line. *Chin Med* 2011; **6**(1): 39.

[6] Chompoo J, Upadhyay A, Fukuta M, Tawata S. Effect of *Alpinia zerumbet* components on antioxidant and skin diseases-related enzymes. *BMC Complement Altern Med* 2012; **12**: 106.

[7] Chompoo J, Upadhyay A, Gima S, Fukuta M, Tawata S. Antiatherogenic properties of acetone extract of *Alpinia zerumbet* seeds. *Molecules* 2012; **17**(6): 6237-6248.

[8] Victório CP, Alviano DS, Alviano CS, Lage CLS. Chemical composition of the fractions of leaf oil of *Alpinia zerumbet* (Pers.) B.L. Burtt & R.M. Sm. and antimicrobial activity. *Rev Bras Farmacogn* 2009; **19**(3): 697-701.

[9] Xuan TD, Fukuta M, Wei AC, Elzaawely AA, Khanh TD, Tawata S. Efficacy of extracting solvents to chemical components of kava (*Piper methysticum*) roots. *J Nat Med* 2008; **62**(2): 188-194.

[10] Clinical and Laboratory Standards Institute. CLSI document M100-S25. *Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement*. Wayne: CLSI; 2015.

[11] Chumpol W, Tavichakorntrakool R, Lulitanond A, Daduang J, Saisud P, Sribenjalux P, et al. The antibacterial activity of the aqueous extract of *Sida acuta* Burm. F. *Southeast Asian J Trop Med Public Health* 2018; **49**(2): 285-291.

[12] Butsat S, Siriamornpun S. Antioxidant capacities and phenolic compounds of the husk, bran and endosperm of Thai rice. *Food Chem* 2010; **119**(2): 606-613.

[13] Costa GM, Suga RG, Oliveira PL, Magalhães PP, Farias LM, Duarte LP, et al. Bioactivity of extracts from *Alpinia zerumbet* (Pers.) B.L. Burtt & R.M. Sm. against sinusitis-causing bacterial pathogens. *Revista Fitos* 2015; **9**(3): 161-252.

[14] Elzaawely AA, Xuan TD, Koyama H, Tawata S. Antioxidant activity and contents of essential oil and phenolic compounds in flowers and seeds of *Alpinia zerumbet* (Pers.) B.L. Burtt. & R.M. Sm. *Food Chem* 2007; **104**(4): 1648-1653.

[15] Barbieri R, Coppo E, Marchese A, Daglia M, Sobarzo-Sánchez E, Nabavi SF, et al. Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. *Microbiol Res* 2017; **196**: 44-68.

[16] Shu Y, Liu Y, Li L, Feng J, Lou B, Zhou X, et al. Antibacterial activity of quercetin on oral infectious pathogens. *Afr J Microbiol Res* 2011; **5**(30): 5358-5361.

[17] Xu HX, Lee SF. Activity of plant flavonoids against antibiotic-resistant bacteria. *Phytother Res* 2001; **15**(1): 39-43.

[18] Xuan T, Teschke R. Dihydro-5, 6-dehydrokavain (DDK) from *Alpinia zerumbet*: Its isolation, synthesis, and characterization. *Molecules* 2015; **20**(9): 16306-16319.

[19] Pham Thi BT. *Pharmacological effects of Alpinia Zerumbet, Momordica charantia and propolis components*. PhD thesis. the United graduate school of Agricultural sciences Kagoshima University; 2016.

[20] Tu PT, Tawata S. Anti-obesity effects of hispidin and *Alpinia zerumbet* bioactives in 3T3-L1 adipocytes. *Molecules* 2014; **19**(10): 16656-16671.

[21] Elzaawely AA, Xuan TD, Tawata S. Changes in essential oil, kava pyrones and total phenolics of *Alpinia zerumbet* (Pers.) B.L. Burtt. & R.M. Sm. leaves exposed to copper sulphate. *Environ Exp Bot* 2007; **59**(3): 347-353.

[22] Elzaawely AA, Xuan TD, Tawata S. Essential oils, kava pyrones and phenolic compounds from leaves and rhizomes of *Alpinia zerumbet* (Pers.) B.L. Burtt. & R.M. Sm. and their antioxidant activity. *Food Chem* 2007; **103**(2): 486-494.