Science and Technology

RMUTT Journal

Vol.8 No.1 (2018): 141-151

www.sci.rmutt.ac.th/stj

Online ISSN2229-1547

Antibacterial activity of medicinal plant extracts against some pathogenic bacteria causing skin diseases

Krongkan Janta[1*] and Watcharaporn Thaharn[2]

[1] Major of Biology, Faculty of Education, Chiang Rai Rajabhat University, Chiang Rai 57100, Thailand

[2] Major of Chemistry, Faculty of Education, Chiang Rai Rajabhat University, Chiang Rai 57100, Thailand

*E-mail: krongkan_jan@hotmail.com

Abstract

This research was aimed to study antibacterial activities of 4 local medicinal plant extracts against 3 species of bacteria causing skin diseases i.e. *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 and methicillin resistant *Staphylococcus aureus* (MRSA). Ethanol and aqueous crude extracts of each plant were prepared to investigate the antibacterial activity by agar well diffusion assay and broth dilution method. It was found that the ethanol extract of *Houttuynia cordata* showed highest activity against *S. aureus* ATCC 25923 and MRSA with MIC of 15.63 and 7.81 mg/ml and MBC of 31.25 and 62.50 mg/ml, respectively. Moreover, the ethanolic extract of *Tiliacora triandra* represented highest growth inhibitory activity on MRSA and *P. aeruginosa* ATCC 27853, at MIC of 7.81 mg/ml and MBC of 125 mg/ml. The cytotoxicity effect of all local medicinal plant extracts on the Vero cells showed that the ethanol extract of *Houttuynia cordata* and *Gymnema inodorum* had the highest toxic effect with CD_{50} of 0.891 mg/ml.

Keywords: medicinal plant, *antibacterial activity*, skin diseases, agar well diffusion, broth dilution

Received: November 26, 2017

Revised: June 22, 2018

Accepted: June 27, 2018

1. Introduction

Many herbal plants have potent medicinal properties and are used as conventional medicines for many disease treatments. It is also safer for patients [1]. Therefore, the trend in the use of medicinal plants is increasing because of their effective properties and high species diversity. Presently, many researchers have been studied on the inhibition effects of some local herbal plants on human pathogen, especially, bacteria.

Thailand is located in the tropical area which is suitable for some bacterial growth. These bacteria are causes of infectious diseases in human, such as respiratory tract disease, gastrointestinal tract disease and skin disease. Skin diseases are normally found in general patients. It is caused by some common bacteria, such as *Staphylococcus epidermidis, Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* (MRSA), *Propioni-bacterium acnes,* and *Pseudomonas aeruginosa*. Generally, bacterial infection can be treated with antibiotics. However, taking antibiotics for long term can cause side effects and also lead to antibiotic drug resistance in patients [2]. Therefore, medicinal plants are another alternative for patients to reduce the risk from antibiotics. Herbal plants are applied to widely use in medicine due to their good treatment results compared to the conventional medicines. Moreover, they are also widely accepted for safety to patients. Many studies have reported the antibacterial activity of crude extracts from some herbs on human skin pathogens. The ethanolic extracts of *Baphicacanthus cusia* [2] showed high activity against *P. aeruginosa*. The ethanolic extract of *Punica granatum* L. var. *granatum* was effective against *S. aureus* [3] while rose (*Rosa damascene* Mill), duzhong (*Eucommia ulmoides* Oliv.) and yerba mate (*Ilex paraguariensis* St. Hill.) showed activity against *P. acnes* [4], The *Aquilaria crassna* leaf extract also had activity against *S. epidermidis* [5]. According to the above mentioned, many reports showed the antibacterial activity of some herbs against pathogenic skin bacteria. However, some of medicinal plants were found only in the local areas and there are rather less data about the activity on skin diseases. Therefore, this research aims to study activity of 4 local medicinal plant extracts against 3 pathogenic bacteria causing skin diseases. The results from this study will be used as based data for further medical or skin products development.

2. Materials and Experiment

2.1 Preparation of crude extracts

Four local medicinal plants, *Houttuynia cordata* Thunb., *Tiliacora triandra* (Colebr.) Diels, *Gymnema inodorum* (Lour.) Decne. and *Gynura procumbens* (Lour.) Merr. were used in this study that were collected from the local area of Chiang Rai Province, Thailand. Fresh leaves were rinsed, dried at 55°C for 24 h and ground to powder. 250 g dried powder of each plant was extracted with 1 L of distilled water or 95% ethanol. The aqueous extracts were placed in a water bath (WNB22, Memmert, Germany) for 3 hours at 45°C. The ethanolic extracts were placed in shaker (LSI-

5002M, Korea) overnight under room temperature. All extracts were filtered through Whatman No.1 and the supernatants were evaporated to increase the concentration using the rotary evaporator (Buchi R-200 rotary evaporator) to remove the solvents. The collected extracts were stored in sterile universal bottles at 2-4 ºC. For further antibacterial activity test, the extracts were dissolved in 100% dimethyl sulfoxide (DMSO; Fisher Scientific) to obtain a concentration of 500 mg/ml.

2.2 Antibacterial assays

Agar well diffusion assay

The antibacterial activity of each crude extract was tested against *S. aureus* ATCC 25923, MRSA and *Pseudomonas aeruginosa* ATCC 27853 derived from Science and Technology Service Center, Faculty of Science, Chiang Mai university (STSC-CMU) using agar well diffusion assay as described by Ismail *et al.* (2016) [6]. Briefly, each bacterial test strain was incubated in 5 ml of Mueller Hinton broth (MHB; Difco Laboratories, USA) at 37 °C for 16-18 hours and the standardized concentration which was equivalent to concentration based on 0.5 McFarland ($1x10^8$ CFU/ml) was prepared which confirmed at OD_{600} nm. Then, each strain was swabbed on Mueller - Hinton agar (MHA; Difco Laboratories, USA). The extracts were dissolved in 100% DMSO to obtain a concentration of 500 mg/ml. 100 µl of the test crude extract was filled into wells (diameter = 6 mm) and incubated at 37 ^{o}C for 24 hours. Gentamicin (BIO BASIC INC.), 1 mg/ml was used for the positive control and 100% DMSO was used as negative control. Then, the bacterial inhibition zone was measured and reported in millimeter (mm). The experiment was determined in triplicates.

Determination of Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The crude extracts which represent positive result were considered to determine MIC value [7]. MIC was defined as the minimal concentration of crude extract which inhibits the growth of bacteria by using broth dilution method. Each extract and gentamicin were prepared by two-fold serial dilution method at concentration of 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, 1.9, 0.9 and 0.4 mg/ml concentration in MHB. Each of bacterial suspension was adjusted to 0.5 McFarland and was inoculated into the tubes containing the extract to obtain 1:1 ratio for determination. Then all tubes were incubated for 24 h at 37 ^{o}C. Gentamicin was used as the positive control. The MIC was determined as the lowest concentration that completely inhibited bacterial growth after 48 h of incubation at 37 ^{o}C. The first tube in the dilution series that showed no growth of bacteria after incubation was defined as MIC. MBC was determined by subculturing from all clear tubes that have no visible growth onto MHA [7] and incubating at 37^{o}C for 48 h. MBC was defined when more than 99.99% of the bacterial inoculum was killed. These determinations were performed in triplicates.

2.3 Cytotoxicity testing using MTT assay [8]

The effect of the ethanolic extracts and the aqueous extracts on the Vero cell (African green monkey, Kidney, that derived from Science and Technology Service Center, Faculty of Science, Chiang Mai university) (STSC-CMU). The Vero cells were cultivated in Dulbecco's modified eagle's medium (D-MEM) (Life Technologies - Gibco) supplemented with 10% fetal bovine serum (FBS) (HyClone, UK). The ethanolic extracts and the aqueous extracts at various concentrations were added into a 96-well culture plate in triplicate and serially diluted ranging from 10 mg/ml to 0.02 mg/ml. The Vero cells (1.5×10^4 cells in 100 µL of media) were seeded into 96-well plates and incubated at 37 ^{o}C in 5% CO_2 incubator (Shellab, USA) for 72 h. The Vero cells growth in DMEM were used as negative control. Cytotoxicity tests were determined by MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide assay) as previously described with modifications by the method of Ali *et al.* (1996) [8]. The growth medium was removed and replaced with MTT reagent (5.0 mg/ml) (Bio Basic, Canada) to each well and incubated for 4 h. Formazan crystals were dissolved by using 100% DMSO (100 µl). The cell control (CC) and vehicle control DMSO was used as the negative control. The absorbance of formazan was measured by a microplate reader (Biochrom, UK) at 570 nm and 630 nm in triplicate. The viable cells will be converted MTT into a purple colored formazan while, died cells did not [9]. The relative percentage of cell viability (%) was expressed to compare with an absorbance of the control well. Analysis of 50% cytotoxic dose (CD_{50}) analysis was determined using probit analysis method [10].

3. Results and Discussions

3.1 Agar well diffusion assay

The results in Table 1 showed antibacterial activity of both the ethanolic and the aqueous extracts. The ethanolic and the aqueous extracts of four local medicinal plant; *Houttuynia cordata* Thunb. (E1), *Tiliacora triandra* (Colebr.) Diels (E2), *Gynura procumbens* (Lour.) Merr. (E3) and *Gymnema inodorum* (Lour.) Decne. (E4) and the aqueous extracts; *Houttuynia cordata* Thunb. (W1), Tiliacora triandra (Colebr.) Diels (W2), *Gynura procumbens* (Lour.) Merr. (W3) and *Gymnema inodorum* (Lour.) Decne. (W4) showed antibacterial activity against *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853 and MRSA.

Table 1 Antibacterial activity of local medicinal plant extracts against pathogenic bacteria

Code	Crude extracts (500 mg/ml)	Inhibition zone diameter (mm)		
		S. aureus **ATCC 25923**	**MRSA**	***P. aeruginosa*** **ATCC 27853**
E1	Ethanolic extracts of *Houttuynia cordata* Thunb.	18.0 ± 0	17.3 ± 0.6	14.3 ± 0.6
E2	Ethanolic extracts of *Tiliacora triandra* (Colebr.) Diels	12.7 ± 0.6	12.3 ± 0.6	12.7 ± 0.6
E3	Ethanolic extracts of *Gynura procumbens* (Lour.) Merr.	13.3 ± 0.6	12.3 ± 0.6	11.0 ± 0
E4	Ethanolic extracts of *Gymnema inodorum* (Lour.) Decne.	NZ	NZ	NZ
W1	Aqueous extracts of *Houttuynia cordata* Thunb.	NZ	NZ	NZ
W2	Aqueous extracts of *Tiliacora triandra* (Colebr.) Diels	NZ	NZ	NZ
W3	Aqueous extracts of *Gynura procumbens* (Lour.) Merr.	NZ	NZ	NZ
W4	Aqueous extracts of *Gymnema inodorum* (Lour.) Decne.	NZ	NZ	NZ
C -	100% DMSO (negative control)	NZ	NZ	NZ
C +	Gentamicin 1 mg/ml (positive control)	32.7 ± 0.6	12.0 ± 0	31.7 ± 0.6

NZ; NZ, No zone of inhibition

The result indicated that the aqueous extract and the ethanolic extracts of *Gymnema inodorum* (Lour.) Decne. cannot inhibit the growth of tested bacteria. For *S. aureus* ATCC 25923, MRSA and *P. aeruginosa* ATCC 27853, the ethanolic extract of *Houttuynia cordata* Thunb. (E1) exhibited the highest zone of inhibition with a diameter of 18.0 ± 0 mm, 17.3 ± 0.6 mm and 14.3 ± 0.6 mm, respectively. The ethanolic extracts of *Tiliacora triandra* (Colebr.) Diels (E2) and *Gynura procumbens* (Lour.) Merr. (E3) showed moderate antibacterial activity with inhibition zones ranging from 12.7-13.3 mm in diameter for *S. aureus* ATCC 25923, 12.3 mm for MRSA and 11.0-12.7 mm for *P. aeruginosa* ATCC 27853. For MRSA, extracts of E1-E3 were found to be more effective than gentamicin 1 mg/ml (control) which showed only 12.0 ± 0 mm in diameter of inhibition zone.

3.2 Minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extracts

Three ethanolic extracts of medicinal plants, (*Houttuynia cordata* Thunb. (E1), *Tiliacora triandra* (Colebr.) Diels (E2) and *Gynura procumbens* (Lour.) Merr. (E3) were considered to determine the MIC and MBC. The results were showed in Table 2.

Table 2 Minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts against pathogenic bacteria

Plant extracts	*S. aureus* ATCC 25923		MRSA		*P. aeruginosa* ATCC 27853	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
E1	15.63	31.25	7.81	62.50	31.25	62.50
E2	15.63	125	7.81	125	7.81	125
E3	15.63	125	31.25	125	62.50	125
Gentamicin	0.50	0.50	8.0	8.0	0.05	0.05

Table 2 represents the ethanolic extract of *Houttuynia cordata* Thunb. (E1) inhibited the growth of *S. aureus* ATCC 25923 at MIC values of 15.63 and 31.25 mg/ml. Moreover, it showed high activity against MRSA at MIC and MBC values of 7.81 and 62.50 mg/ml, respectively. The ethanolic extract of *Tiliacora triandra* (Colebr.) Diels (E2) represented the antimicrobial activity against MRSA with the MIC of 7.81 mg/ml and MBC of 125 mg/ml. It also had the greatest inhibitory activity against *P. aeruginosa* ATCC 27853, with MIC of 7.81 mg/ml and MBC of 125 mg/ml.

According to the results of the antimicrobial activity of crude extract from local medicinal plants, more efficiency against Gram-positive bacteria (*S. aureus* and MRSA) was found compared to Gram-negative bacteria (*P. aeruginosa*). These results agreed with the report of Martinez de Tejada *et al.* (2012) that it was due to differences of the cell wall structure between Gram-negative bacteria and Gram-positive bacteria. Cell wall of Gram-negative bacteria is composed of the outer membrane containing the layer of lipopolysaccharide (LPS) which is an impermeable barrier to all charged molecules. In the other hand, Gram-positive bacteria lack an outer membrane which makes it unable to resist the penetration of antimicrobial substances [11]. In addition, the aqueous extract of four plants had no antibacterial activities on the tested bacteria, while the ethanolic extracts presented higher inhibitory effect due to extraction ability of the examined solvent. This result was consistent with previous studies of Witkowska *et. al.* (2013) and Gberikon *et al.* (2015) which reported that alcoholic solvents (ethanol and methanol) were more suitable than water for extracting bioactive compounds from medicinal plants [12-13]. The bioactive compounds of some plants in this study had previously been studied and reported by several researchers. For examples, Fu *et al*. (2013) reported that the ethanol extract of *Houttuynia cordata* Thunb showed the presence of

some bioactive compounds that were the type of methyl-ester. This compound could inhibit some pathogen bacteria, such as *Burkholderia pseudomallei*, *Shigella flexneri*, *Salmonella* group B, *Staphylococcus aureus* and Group A streptococci [14]. Furthermore, Ahmed *et al.* (2016) found that the ethanol extract of *Houttuynia Cordata* had inhibitory effect on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* [15].

Another herbal plant, *Tiliacora triandra* (Colebr.) Diels. presented some groups of bioactive compounds. The volatile oil from the leaves of *Tiliacora triandra* (Colebr.) Diels consisted of β-Linalool, ∝-terpineol and β-ionone. These compounds presented antimicrobial activities against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Salmonella* sp. as reported by Naibaho *et al.* (2012) and Klinklan *et al*. (2014) [16-17]

In addition, in another study by Rahman and Asad (2013) reported that *Gynura procumbens* (Lour.) Merr. exhibited antibacterial activity against some Gram-positive and Gram-negative bacteria such as *Bacillus cereus, P. aeruginosa, Vibrio parahaemolyticus* and *Salmonella typhi*. [18]. *Gynura procumbens* had activity against *S. aureus* and resistant strains, dysentery bacillus, typhoid bacillus, paratyphoid bacillus, *Escherichia coli* and *P. aeruginosa* [19]. Some bioactive compound groups, such as phenolic content and flavonoid content were found in *Gynura procumbens* (Lour.) Merr. [20]. Therefore, these compounds as mentioned above from previously reports that were found in the plant extracts in this research (*Houttuynia Cordata, Tiliacora triandra* (Colebr.) and *Gynura procumbens* (Lour.) Merr.) could also affect the tested bacteria.

3.3 Cytotoxicity of plant extracts

The toxicity of ethanolic plant extracts and aqueous plant extracts to the Vero cell were presented in Table 3. The cell control (CC) was tested and it showed 100% cell viability. The 0.5% DMSO which was used as a negative control, had no toxicity to the Vero cell. The aqueous extract of *Tiliacora triandra* (Colebr.) Diels (W2) and *Gynura procumbens* (Lour.) Merr. (W3) was found to have the lowest toxicity with 50% viability of cells (CD_{50}) at concentrations of 3.548 ± 0.000 mg/ml, whereas the ethanolic extract of *Houttuynia cordata* Thunb. (E1) and *Gymnema inodorum* (Lour.) Decne. (E4) were found to be the most toxic with CD_{50} value of 0.891 ± 0.000 mg/ml.

Table 3 The toxicity of ethanolic plant extracts and aqueous plant extracts to the Vero cell

Code	Sample	CD_{50} (mg/ml)
E1	Ethanolic extracts of *Houttuynia cordata* Thunb.	0.891 ± 0.000
E2	Ethanolic extracts of *Tiliacora triandra* (Colebr.) Diels	1.483 ± 0.512
E3	Ethanolic extracts of *Gynura procumbens* (Lour.) Merr.	1.778 ± 0.000
E4	Ethanolic extracts of *Gymnema inodorum* (Lour.) Decne.	0.891 ± 0.000
W1	Aqueous extracts of *Houttuynia cordata* Thunb.	1.714 ± 0.112
W2	Aqueous extracts of *Tiliacora triandra* (Colebr.) Diels	3.548 ± 0.000
W3	Aqueous extracts of *Gynura procumbens* (Lour.) Merr.	3.548 ± 0.000
W4	Aqueous extracts of *Gymnema inodorum* (Lour.) Decne.	1.778 ± 0.000

According to results shown in Table 3, the ethanolic extracts of *Houttuynia cordata* Thunb. (E1) and *Gymnema inodorum* (Lour.) Decne. (E4) showed highest toxicity. However, it was analyzed only by an initial screening method using the Vero African green monkey kidney cell lines. For further investigation, the cytotoxicity test should be conducted on animals and human skin or the dermal irritation and skin sensitization using laboratory animal *tests* were needed to confirm the effect of extracts. In addition, further studies are needed to determine the optimal concentration of crude extracts when used as the ingredients in pharmaceuticals product or skin care products.

4. Conclusions

The antibacterial activities of four local medicinal plant crude extracts, *Tiliacora triandra* (Colebr.) Diels, *Houttuynia cordata* Thunb., *Gymnema inodorum* (Lour.) Decne. and *Gynura procumbens* (Lour.) Merr. were tested against *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923 and MRSA. The ethanolic extracts of *Houttuynia cordata* Thunb. (E1) showed the highest activity with MIC and MBC values of 15.63 and 31.25 mg/ml against *S. aureus* ATCC 25923 and with MIC and MBC values of 7.81 and 62.50 mg/ml against MRSA respectively. The ethanolic extract of *Tiliacora triandra* (Colebr.) Diels had the

greatest inhibitory activity against *P. aeruginosa* ATCC 27853, with MIC of 7.81 mg/ml and MBC of 125 mg/ml. However, all aqueous extracts and the ethanolic extracts of *Gymnema inodorum* (Lour.) Decne. had no inhibitory effect against tested bacteria. The ethanolic extracts of *Houttuynia cordata* Thunb. (E1) and *Gymnema inodorum* (Lour.) Decne. (E4) had the highest cytotoxicity with CD_{50} of 0.891 mg/ml.

According to these results showing the antimicrobial activities of the four local plants on tested bacteria that causing skin diseases (*S. aureus* ATCC 25923, MRSA and *P. aeruginosa* ATCC 27853), they will be the basis data for further studies on local plants investigated, to determine their antioxidant activity and their cytotoxicity in animals and human for possibility on skin product development and added value to the local herbal plants. These results suggested that the ethanolic extracts of *Houttuynia cordata* Thunb. (E1) and *Tiliacora triandra* (Colebr.) Diels showed the highest activity on tested bacteria. They may use as preliminary information and could be further studied for uses in pharmaceutical and skin care products applications.

5. Acknowledgements

The work was funded and supported by The Research and Development Institute, Chiang Rai Rajabhat University. The authors thank the faculty of science and technology, Chiang Rai Rajabhat University for support the laboratory during this study.

The tested bacteria, *S. aureus* ATCC 25923, MRSA and *P. aeruginosa* ATCC 27853 derived from Science and Technology Service Center, Faculty of Science, Chiang Mai university (STSC-CMU) and cytotoxicity testing using MTT assay was also analyzed by STSC-CMU.

6. References

[1] Nasri H. and Shirzad H. Toxicity and safety of medicinal plants. *J. Herbmed. Plarmacol*. 2013. 2(2): 21-22.

[2] Chaichompool A. and Thongwai N. Growth inhibition of some pathogenic bacteria by local medicinal plant extracts. *Proceeding of the 8th Kasetsart University Kamphaeng Saen Campus conference*, Thailand. 8-9 December 2011, 792-801.

[3] Chansakaow S., Leelapornpisid P., Yosprasit K. and Tharavichitkul P. Antibacterial activity of Thai medicinal plant extracts on the skin infectious microorganisms. In III WOCMAP Congress on Medicinal and Aromatic Plants. *Targeted Screening* of MAPs, *Economics* & Law. 2003. 678(4) : 153-157.

[4] Tsai T., Wu W., Tseng J.T. and Tsai P.J. *In vitro* antimicrobial and anti-inflammatory effects of herbs against *Propionibacterium acnes*. *Food Chem*. 2010. 119(3) : 964-968.

[5] Kamonwannasit S., Nantapong N., Kumkrai P., Luecha P., Kupittayanant S. and Chudapongse N. Antibacterial activity of *Aquilaria crassna* leaf extract against *Staphylococcus epidermidis* by disruption

of cell wall. *Ann. Clin. Microbiol. Antimicrob.* 2013: 12(1): 20.

[6] Ismail Y., Wedyan M., Al-Zu'abe M. and Abderrahman S. Antimicrobial activity of *Rubia cordifolia*: methods to determine antimicrobial activity. *J. Med. Plants Res.* 2016. 10(8): 457-462.

[7] Rios J.L, Recio MC and Villar A. Screening methods for natural products with antimicrobial activity. *J. Ethnopharmacol.* 1988. 23(2): 127-49.

[8] Ali A.M., Mackeen M.M. and El - Sharkawy S.H. Antiviral and cytotoxic activities of some plants used in Malaysian indigenous medicine. *Pertanika J. Trop. Agric. Sci.* 1996. 19 (2-3): 129-136.

[9] Marshall N.J., Goodwin C.J. and Holt S.J. A critical assessment of the use of microculture tetrazolium assays to measure cell growth and function. *J. Plant Growth Regul.* 1995. 5(2): 69-84.

[10] Finney D.J. Probit Analysis. 3rd Ed., Cambridge University Press, London. 1971.

[11] Martinez de Tejada G., Sánchez-Gómez S., Rázquin-Olazaran I., Kowalski I., Kaconis Y., Heinbockel L., Andrä J., Schürholz T., Hornef M., Dupont A., Garidel P., Gutsmann T., David S.A., Brandenburg K. and Garidel, P. Bacterial cell wall compounds as promising targets of antimicrobial agents I. Antimicrobial peptides and lipopolyamines. *Curr. Drug Targets*. 2012. 13(9) : 1121-1130.

[12] Witkowska A.M., Hickey D.K., Alonso-Gomez M. and Wilkinson M. Evaluation of antimicrobial activities of commercial herb and spice extracts against selected food-borne bacteria. *J. Food Res.* 2013. 2(4) : 37-54.

[13] Gberikon G.M., Adeoti I.I. and Aondoackaa A.D. Effect of ethanol and aqueous solutions as extraction solvents on phytochemical screening and antibacterial activity of fruit and stem bark extracts of *Tetrapleura tetrapteraon Streptococcus salivarus* and *Streptococcus mutans*. *Int. J. Curr. Microbiol. App. Sci.* 2015. 4(5): 404-410.

[14] Fu J., Dai L., Lin Z. and Lu H. *Houttuynia cordata* Thunb: a review of phytochemistry and pharma- cology and quality control. *Chin. Med. J*. 2013. 4(3): 101.

[15] Ahmed S., Borah M. and Das S. A comparative study of the antibacterial activity of the ethanolic extracts of *Phlogacanthus Thyrsifloru.*, *Houttuynia cordata*, *Curcuma caesia* and *Syzygium jumbos*. *Int. J. Pharm*. 2012. 3(9) : 282-285.

[16] Naibaho N.M., Kerdchoechuen O. and Laohankunjit N. Physico-chemical properties of plant extracts from Yanang *(Tiliacora triandra)* leaves. *J. Agric. Sci.* 2012. 43(2): 533-536.

[17] Klinklan K., Kerdchoechuen O. and Laohankunjit N. Volatile compounds and antibacterial activity of starch foam coated herbs essential oils. *J. Agric. Sci.* 2014. 45(2): 705-708.

[18] Rahman A.F. and Asad M. Chemical and biological investigations of the leaves of *Gynura procumbens*. *Int. J. Biosci*. 2013. 3(4): 36-43.

[19] Xie P. Skin care creams containing *Gynura* Extracts. CN. Patent No. 101032458. Beijing: State Intellectual Property Office of the P.R.C. 2007.

[20] Tan H.L., Chan K.G., Pusparajah P., Lee L.H., and Goh B.H. *Gynura procumbens*: an overview of the biological activities. *Front. Pharmacol.* 2016. 7: 52.