J. of Korean Oil Chemists' Soc.,
Vol. 33, No. 2. June, 2016. 278~285
ISSN 1225-9098 (Print)
ISSN 2288-1069 (Online)
http://dx.doi.org/10.12925/jkocs.2016.33.2.278

Antibacterial Activity of *Suaeda australis* in Halophyte

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(Received May 9, 2016; Revised June 15, 2016; Accepted June 15, 2016)

Abstract : The discovery of various activities of natural plants has increased interest in halophytes. *Suaeda australis* and *S. maritime* are perennial halophytes that belong to the Chenopodiaceae family. Extracts of *S. australis* and *S. maritime* plants were investigated for concentration and time-dependent antibacterial and antioxidant activities using bacterial species and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, respectively, as well as total phenolic content. The *S. australis* extract (500 μg/mL) showed activity against all the bacterial species including *P. aeruginosa*, *P. mirabilis*, *A. baumannii*, and VRE with 61.1, 42.3, 44.49, and 40.38%, respectively, inhibition and suppressed of these four species for 12 h. Overall, the *S. australis* extract showed marked antibacterial activities while, in contrast, the *S. maritime* extract had excellent antioxidant effects. However, the effects of the two extracts were much lower than that of quercetin. The present study identified antibacterial activities of *S. australis*, and it would be necessary to perform further phytochemical studies of *S. australis*.

Keywords : Suaeda australis, Suaeda maritime, antibacterial activity, antioxidant activity

1. Introduction

In 1928, Fleming discovered that blue mold grew on plates where *Staphylococcus* species were cultured, and cells around the fungi were killed, which led to the discovery of penicillin, the first antibiotic[1]. Since then, life-threatening infections such as bacteremia, bacterial meningitis, tuberculosis and pneumonia have been treated with antibiotics[2]. However, some pathogenic bacteria have acquired resistance to antibiotics owing to continuous abuse of broad ranges of antibiotics[3]. This has led to the emergence of pathogenic bacteria that are resistant to various antibiotics, which has created a major medical problem worldwide and a significant threat to human health[4].

Hospital infections are considered the biggest problem in medical facilities worldwide and lead to the death of patients, failure of surgeries, graft rejections, failure of chemotherapy, increased hospital expense burden for patients, and long-term

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hospitalization[5]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is classified as a causative pathogen of hospital infections and has been identified as one of the most common bacterial pathogens since its discovery in 1961. In addition, recent studies indicate that MRSA infections still are attributable for serious negative clinical outcomes despite antibacterial treatment[6]. Furthermore, MRSA accounts for 60-70% of hospital infections caused by *Staphylococcus aureus* in Korea, and the use of glycopeptide antibiotics such as vancomycin for the treatment of MRSA has increased[7,8]. This has gradually increased the number of pathogens that are resistant to antibiotics.

The World Health Organization (WHO) reported that natural plants would be useful in the treatment of diseases caused by antibiotic-resistant bacteria, and interest in natural resources with useful bioactivities against infectious diseases has increased in recent years[9]. The various natural plants, interest has increased considerably in halophyte plants, which are resistant to high soil salt levels, and are found in sand dunes, rocky coasts, saline depressions, inland deserts, and coastal environments such as salt marshes of the coast[10]. It is known that halophytes thrive in and are resistant to these harsh environments[11]. In addition, they can inhabit a broad range of environments and are equipped with an antioxidant system centered on their phenolic compound content[12]. The human body possesses a defense system with antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which function to prevent oxidative damage[13]. However, this system can lose its effectiveness owing to structural and functional modifications caused by excessive production of free radicals[14]. Therefore, there is a need to protect the body from oxidative stress using the natural antioxidants.

Suaeda australis and *S. maritime* are perennial halophytes that belong to the family Chenopodiaceae and are distributed in the West and Southern coast, Jeju Island in Korea, Southern China, Taiwan, and Southern Japan. The seven genera of Chenopodiaceae reported in Korea, the *Suaeda* species, which are regarded as the most important, were studied for various folkloric medical effects including hypoglycemic and hypolipidemic (*S. fruticose*), antimicrobial (*S. pruinosa*), and anti-oxidative and anti-inflammatory (*S. asparagoides*) [15-18]. In contrast, few studies have investigated the effects of *S. australis* and *S. maritime* thus far. Therefore, the present study aimed to investigate the antibacterial and antioxidant effects of extracts of *S. australis* and *S. maritime* against various bacterial species, to provide basic data for the development of natural antibiotics that could replace currently used synthetic antibiotics.

2. Materials and Methods

2.1 Extraction and isolation of plant constituents

The whole plants of *S. australis* and *S. maritime*, which grow wild in West coast were extracted thrice with ethanol (EtOH) at room temperature (1 day per each extraction), and the extracts were vacuum-dried at 40° C followed by resuspension in EtOH to a concentration of 10 mg/mL for further use in the tests. Quercetin (Sigma-Aldrich, USA) was used as a positive control in the determination of antioxidant effects.

2.2 Bacterial strains

The bacterial strains used to investigate the specific antibacterial activities of extracts of *S. australis* and *S. maritime* included the gram-positive *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* (ATCC 19615), *Enterococcus faecalis* (ATCC 19433), *Listeria monocytogenes* (ATCC 15313), and *Bacillus cereus* (KCTC 1014); gram-negative

Pseudomonas aeruginosa (ATCC 27853), *Proteus mirabilis* (ATCC 7002), *Klebsiella pneumonia* (ATCC 13883), *Acinetobacter baumannii* (ATCC 19606), and *Neisseria gonorrhoeae* (Clinical Isolates); and antibiotic-resistant methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), and multidrug-resistant *Pseudomonas aeruginosa* (MDR-PA). These strains were spread on blood agar plate (BAP) and then cultured in brain heart infusion (Difco, USA) broth overnight prior to being used.

2.3 Concentration-dependent extract antibacterial activities

The bacterial suspensions cultured in BHI media were further diluted to 0.5×10^5 cell/mL with BHI and the optical densities were measured at 600 nm. The natural plant extracts were serially diluted to 250 and 500 μg/mL with BHI medium, aliquoted into a 96-well plate (100 μL per well), and then the same volume of diluted bacterial suspensions was added to each well, based on the strain. The plate was then cultured in an incubator at 37° C for 24 h and the negative control was BHI liquid medium. Following the incubation, optical densities were measured at 600 nm using Biotrak Ⅱ Plate reader (Amersham Life Science, UK).

2.4 Time-dependent extract antibacterial activities

Antibacterial activity testing was carried out by using the above method. The natural plant extracts were diluted with BHI medium to a concentration of 500 μg/mL and the same volume of each diluted bacterial suspension was added, followed by incubation in an incubator at 37° C for 2, 4, 8, 12 and 24 h. The optical densities were measured at 600 nm using Spectronic Genesys 5 (Milton Roy Company, USA).

2.5 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The radical scavenging activities of the extracts of *S. australis* and *S. maritime* were measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay[19]. The natural plant extracts were prepared as 1 and 5 mg/mL dilutions in each solvent, aliquoted to a 96-well plate with 10 μL per well, and then 190 μL of 200 μM DPPH (Sigma-Aldrich, USA) in ethanol was added to each well. This was followed by incubation at 37° C for 30 min, and then the optical densities were measured at 550 nm using Biotrak Ⅱ Plate reader (Amersham Life Science, UK).

2.6 Total phenolic content

The total phenolic content of the extracts was measured using the Folin-Denis method with modifications[20]. Briefly, 50 μL of the extracts at 1 mg/mL concentration in distilled water (DW, 1.65 mL), and 100 μL Folin-Denis reagent (Sigma-Aldrich, USA) were mixed for 5 min, and then 200 μL 1N sodium carbonate (Na_2CO_3) was added. This was followed by incubation at room temperature for 2 h, and then the optical density was measured at 750 nm using Spectronic Genesys 5 (Milton Roy Company, USA). Gallic acid was subjected to the same procedure as the samples as a standard to obtain a standard curve, which was used to determine the total phenolic contents that were expressed as gallic acid equivalents per gram of extract sample (mg GAE/g).

3. Results and discussion

3.1 Concentration-dependent extract antibacterial activities

Well-known halophytes to date include *Spergularia marina*, *Limonium tetragonum*, and *Salicornia herbacea*[21-23]. The antibacterial activities of the extracts of *S. australis* and *S.*

maritime were shown to be concentration-dependent. As shown in Table 1, The *S. australis* extract at 500 μg/mL (mean of three replicates) inhibited the gram-positive bacteria (*S. aureus, S. pyogenes, E. faecalis, L. monocytogene,* and *B. cereus*) by 36.40, 32.8, 16.48, 34.44, and 29.2%, respectively; the extract also inhibited the growth of gram-negative bacteria such as *P. aeroginosa*, *P. mirabilis*, *K. pneumonia*, *A. baumannii*, *N. gonorrhoeae* by 61.1, 42.3, 31.5, 44.49 and 22.91%, respectively. In addition, the *S. australis* extract inhibited antibiotic-resistant bacteria at 25.4, 40.38, and 38.37% for MRSA, VRE, and MDR-PA, respectively. Taken together, the *S. australis* extract showed activity against the gram-positive, gram-negative, and antibiotic-resistant bacteria tested. The *S. maritime* extract at 500 μg/mL, inhibited the gram-positive bacteria at 67.7, 32.08, 36.63, and 16.06% for *S. aureus*, *S. pyogenes*, *L. monocytogene*, and *B. cereus*, respectively, while gram-negative bacterial inhibition was 59.08, 39.32, 20.95, and 37.67% for *P. aeroginosa*, *P. mirabilis*, *K. pneumonia*, and *A. baumannii*, respectively. In addition, *S. maritime* extract inhibited the antibiotic-resistant bacteria at 13.3, 41.07, and 31.74% for MRSA, VRE, and MDR-PA, respectively. Although *S. maritime* extract showed activity against all bacterial species tested except *E. faecalis* and *N. gonorrhoeae*, the *S. australis* extract showed an overall higher antibacterial activity.

3.2 Time-dependent extract antibacterial activities

As shown in Fig. 1, The *S. australis* extract at 500 μg/mL, showed time-dependent (2, 4, 8, 12, and 24 h) antibacterial activity against

Table 1. Antibacterial activity of extracts from *S. australis* and *S. maritime*

strain	*S. australis*		*S. maritima*	
	Concentration (μg/mL)		Concentration (μg/mL)	
	250	500	250	500
S. aureus	10.02	36.4	4.5	**67.7***
S. pyogenes	15.72	32.8	11.32	32.08
E. faecalis	7.18	16.48	NA	NA
L. monocytogenes	12.86	34.44	10.46	36.63
B. cereus	NA	29.2	13.74	15.06
P. aeruginosa	19.27	**61.1***	12.7	**59.08***
P. mirabilis	5.32	**42.34***	9.49	39.32
K. pneumoniae	8.06	31.5	6.05	20.95
A. baumannii	12.46	**44.49***	NA	37.67
N. gonorrhoeae	18.19	22.91	NA	NA
MRSA	17.33	25.4	1.9	13.3
VRE	28.55	**40.38***	17.24	**41.07***
MDR-PA	20.79	38.37	NA	31.74

* : More than 40% anti-bacterial effect, NA : without antibacterial inhibitory effect, MRSA : Methicillin resistant *staphylococcus aureus,* VRE : Vancomycin-resistant *enterococci,* MDR-PA : Multidrug-resistant *Pseudomonas aeruginosa*. These results were obtained by three independent experiments.

four bacterial strains including *P. aeruginosa*, *P. mirabilis*, *A. baumannii*, and VRE. All four strains cultured in the absence of the extract showed bacterial growth after 4 h, whereas there was no growth even 12 h following treatment with the *S. australis* extract; however, slight bacterial growth was observed after 12 h. *S. australis* extract showed activity against all the species tested and, in particular, inhibited the gram-negative *P. aeruginosa* by 60%. *P. aeruginosa* is an important causative bacterium in a number of hospital infections including pneumonia, urinary tract infections, gastrointestinal infections, and sepsis[24]. In addition, the number of antibiotic-resistant bacterial strains has increased due to continuous use of antibiotics, leading to more serious and difficult to treat infections. The *S. australis* extract showed an inhibitory activity of more than 38% against MDR-PA in addition to its effects against *P. aeruginosa*. Although the *S. maritime* extract had an overall lower antibacterial activity than *S. australis* did, it had a higher antibacterial activity against *S. aureus*. Most hospital- and community-acquired infections are caused by *S. aureus*, and the increased antibiotic resistance has become a serious problem worldwide[25]. The extracts of *S. australis* and *S. maritime* showed activities against bacteria that are the major causes of antibiotic-resistant infections and, therefore, they can considered as good potential candidates to replace synthetic antibiotics.

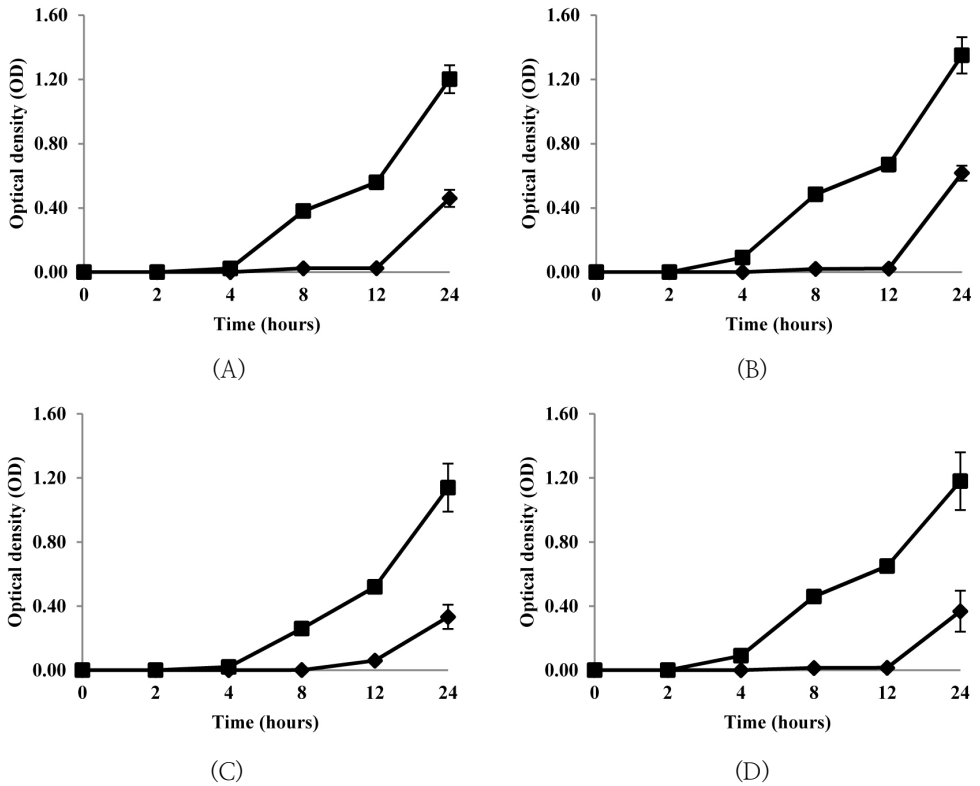

Fig. 1. Inhibition of bacteria growth by *S. australis* extracts on time. (A) *Pseudomonas aeruginosa*, (B) *Proteus mirabilis*, (C) *Acinetobacter baumannii*, (D) Vancomycin-resistant *enterococci*, ◆, 500 μg/mL; ■, Negative control. These results were obtained by three independent experiments.

Further studies to elucidate the specific constituents of both these natural plant extracts are necessary.

3.3. DPPH radical scavenging activity

Although halophytes are known to contain compounds including vitamins, polysaccharides, glycosides, and phenols[26]. The DPPH radical scavenging activity test was performed to evaluate the antioxidant effects of the *S. australis* and *S. maritime* extracts. As shown in Fig. 2, the *S. australis* extract showed slight antioxidant effects of less than 1 and 7.25% at 1 and 5 mg/mL, respectively. In contrast, the *S. maritime* extract showed antioxidant effects of 8.01 and 25.26% at 1 and 5 mg/mL, respectively. Although the *S. maritime* extract showed a higher antioxidant effect than the *S. australis* extract did, both extracts showed lower effects than quercetin.

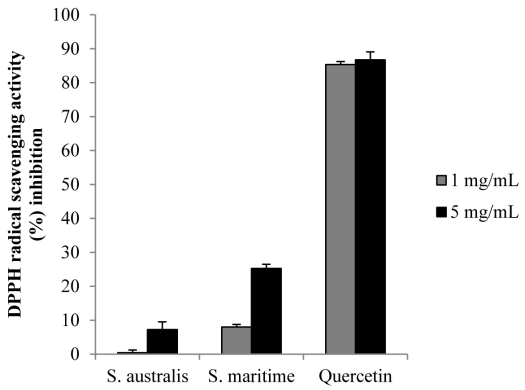

Fig. 2. DPPH radical scavenging activity of extracts from *S. australis*, *S. maritime* and quercetin. These results were obtained by three independent experiments.

3.4 Total phenolic content

As shown in Table 2, Total phenolic contents were determined to be 7.87 and 24.47 mg GAE/g of *S. australis* and *S. maritime* extracts, respectively. Riadh *et al.* measured the phenolic content of the halophyte *Tamarix gallica,* and its leaves and flowers were shown to contain 34.44 mg and 135.35 mg GAE/g of extract, respectively[27]. This result indicates a higher phenolic content for *T. gallica* than for the *S. australis* and *S. maritime* extracts.

Table 2. Total phenolic content of extracts from *S. australis* and *S. maritime*

Extracts	Total phenolic content (mgGAE/gextract)
S. australis	7.87±0.61
S. maritima	24.47±4.64

4. Conclusions

Halophytes are plants that have gained attention recently and are known to have high phenolic contents and various biological activities. There are currently few studies on the halophytes *S. australis* and *S. maritime* and, therefore, the present study compared and evaluated the antibacterial and antioxidant effects of their extracts. The antibacterial activities of the extracts of *S. australis* and *S. maritime* were shown to be concentration and time-dependent. However, DPPH radical-scavenging activities of the *S. australis* and *S. maritime* extracts were not outstanding in this study. The present study demonstrated the antibacterial activities of *S. australis* and *S. maritime* and based on these results, it would be expedient to conduct further phytochemical analysis of these extracts.

Acknowledgement

This work was supported by the Brain Busan 21 Project in 2016.

References

1. A. G. Browne, M. C. Fisher, and D. A. Henk, Species-specific PCR to describe local-scale distributions of four cryptic species in the Penicillium chrysogenum complex, *Fungal Ecol.*, **6(5)**, 419-429 (2013).
2. A. M. Schubert, M. A. Rogers, C. Ring, J. Mogle, J. P. Petrosino, V. B Young, D. M. Aronoff, and P. D. Schloss, Microbiome data distinguish patients with Clostridium difficile infection and non-C. difficile-associated diarrhea from healthy controls, *MBio.*, **5(3)**, e01021-14(2014).
3. K. Priya, W. F. Yin, and K. G. Chan, Anti-quorum sensing activity of the traditional Chinese herb, *Phyllanthus amarus*, Sensors (Basel), **13(11)**, 14558-14569(2013).
4. B. K. Dash, M. K. Sen, K. Alam, K. Hossain, R. Islam, N. A. Banu, S. Rahman, and A. H. Jamal, Antibacterial activity of *Nymphaea nouchali* (Burm. f) flower, *Ann Clin Microbiol Antimicrob.*, **12(27)**, 1-4(2013).
5. S. Behzadnia, A. Davoudi, M. S. Rezai, and F. Ahangarkani, Nosocomial infections in pediatric population and antibiotic resistance of the causative organisms in north of iran, *Iran Red Crescent Med J.*, **16(2)**, e14562(2014).
6. J. Kos, I. Zadrazilova, M. Pesko, S. Keltosova, J. Tengler, T. Gonec, P. Bobal, T. Kauerova, M. Oravec, P. Kollar, A. Cizek, K. Kralova, and J. Jampilek, Antibacterial and herbicidal activity of ring-substituted 3-hydroxynaphthalene-2-carboxanilides. *Molecules*, **18(7)**, 7977-7997(2013).
7. E. S. Kim, J. S. Song, H. J. Lee, P. G. Choe, K. H. Park, J. H. Cho, W. B. Park, S. H. Kim, J. H. Bang, D. M. Kim, K. U. Park, S. Shin, M. S. Lee, H. J. Choi, N. J. Kim, E. C. Kim, M. D. Oh, H. B. Kim, and K. W. Choe, A survey of community-associated methicillin-resistant Staphylococcus aureus in Korea. *J Antimicrob Chemother*, **60(5)**, 1108-1114 (2007).
8. J. H. Hwang, J. H Lee, M. K. Moon, J. S. Kim, K. S. Won, and C. S. Lee, The efficacy and safety of arbekacin and vancomycin for the treatment in skin and soft tissue MRSA infection: preliminary study. *Infect Chemother.*, **45(1)**, 62-68 (2013).
9. M. L. Bastos, M. R. Lima, L. M. Conserva, V. S. Andrade, E. M. Rocha, and R. P. Lemos, Studies on the antimicrobial activity and brine shrimp toxicity of *Zeyheria tuberculosa* (Vell.) Bur. (Bignoniaceae) extracts and their main constituents. *Ann Clin Microbiol Antimicrob.*, **8(16)**, 1-6(2009).
10. M. J. Rodrigues, K. N. Gangadhar, C. Vizetto-Duarte, S. G. Wubshet, N. T. Nyberg, L. Barreira, J. Varela, and L. Custódio, Maritime halophyte species from southern Portugal as sources of bioactive molecules. *Mar Drugs.*, **12(4)**, 2228-2244 (2014).
11. C. S. Kong, Anti-Inflammatory Activity of the Solvent-Partitioned Fractions from *Spergularia marina* in LPS-Stimulated RAW 264.7 Cells, *Prev Nutr Food Sci.*, **19(4)**, 261-267(2014).
12. F. Zar Kalai, J. Han, R. Ksouri, A. El Omri, C. Abdelly, and H. Isoda, Antiobesity Effects of an Edible Halophyte Nitraria retusa Forssk in 3T3-L1 Preadipocyte Differentiation and in C57B6J/L Mice Fed a High Fat Diet-Induced Obesity, *Evid Based Complement Alternat Med.*, **2013**, 368658 (2013).
13. T. Debnath, S. R. Park, D. H. Kim, J. E. Jo, and B. O. Lim, Anti-oxidant and anti-inflammatory activities of *Inonotus obliquus* and germinated brown rice extracts. *Molecules*, **18(8)**, 9293-9304 (2013).

14. C. T. Kumarappan, E. Thilagam, and S. C. Mandal, Antioxidant activity of polyphenolic extracts of *Ichnocarpus frutescens*, *Saudi J Biol Sci.*, **19(3)**, 349-355(2012).
15. S. C. Choi, S. H. Lim, S. H. Kim, D. G. Choi, J. G. Kim and Y. S. Choo, Growth and solute pattern of *Suaeda maritima* and *Suaeda asparagoides* in an abandoned salt field, *J Ecol Field Biol.*, **35(4)**, 351-358 (2012).
16. W. A. Tawfik, M. M. Abdel-Mohsen, H. M. Radwan, A. A. Habib, and M. A. Yeramian, Phytochemical and biological investigations of *Atriplex semibacata* R .BR. growing in Egypt, *Afr J Tradit Complement Altern Med.*, **8(4)**, 435-443 (2011).
17. S. Oueslatia, N. Trabelsia, M. Boulaabaa, J. Legaultb, C. Abdellya, and R. Ksouria, Evaluation of antioxidant activities of the edible and medicinal Suaeda species and related phenolic compounds. *Industrial Crops and Products*, **36(2012)**, 513-518 (2011).
18. J. M. Park, S. D. Kim, W. M. Lee, J. Y. Cho, H. J. Park, T. W. Kim, N. H Choe, S. K. Kim, and M. H. Rhee, In vitro anti-oxidative and anti-inflammatory effects of solvent-extracted fractions from *Suaeda asparagoides*. *Pharmazie.*, **62**, 453-458(2007).
19. H. R. Kim, G. N. Park, B. K. Jung, W. J. Yoon, Y. H. Jung, and K.S. Chang, Antioxidative Effects of *Rhaphiolepis indica* and *Quercus salicina* from Jeju. *J. of Korean Oil Chemists' Soc.* **33(1)**, 41-50(2016).
20. A. A. Bellail, O. E. Shaltout, M. M. Youssef, and A. A. El-Gamal, Effect of Home-Cooking Methods on Phenolic Composition and Antioxidant Activity of Sweetpotato (*Ipomoea batatas* (L.) Lam.) Cultivars Grown in Egypt. *Food and Nutrition Sciences*, **3(4)**, 490-499(2012).
21. F. Karadeniz, J. A. Kim, B. N. Ahn, M. Kim, and C. S. Kong, Anti-adipogenic and Pro-osteoblastogenic Activities of *Spergularia marina* Extract, *Prev Nutr Food Sci.*, **19(3)**, 187-193(2014).
22. M. H. Yang, N. H. Kim, J. D. Heo, S. H. Sung, and E. J. Jeong, Hepatoprotective effects of *Limonium tetragonum*, edible medicinal halophyte growing near seashores, *Pharmacogn Mag.*, **10(3)**, S563-568(2014).
23. J. H. Sung, S. H. Park, D. H. Seo, J. H. Lee, S. W. Hong, and S. S. Hong, Antioxidative and skin-whitening effect of an aqueous extract of *Salicornia herbacea*, *Biosci Biotechnol Biochem.*, **73(3)**, 552-556(2009).
24. S. Y. Lee, Y. J. So, M. S. Shin, J. Y. Cho, and J. Lee, Antibacterial effects of afzelin isolated from *Cornus macrophylla* on *Pseudomonas aeruginosa*, a leading cause of illness in immunocompromised individuals, *Molecules*, **19(3)**, 3173-3180 (2014).
25. Y. S. Kim, H. Kim, E. Jung, J. H. Kim, W. Hwang, E. J. Kang, S. Lee, B. J. Ha, J. Lee, and D. Park, A novel antibacterial compound from *Siegesbeckia glabrescens*, *Molecules*, **17(11)**, 12469-12477(2012).
26. R. Ksouri, W. M. Ksouri, I. Jallali, A. Debez, C. Magné, I. Hiroko, and C. Abdelly, Medicinal halophytes: potent source of health promoting biomolecules with medical, nutraceutical and food applications, *Crit Rev Biotechnol.*, **32(4)**, 289-326(2012).
27. R. Ksouri, H. Falleh, W. Megdiche, N. Trabelsi, B. Mhamdi, K. Chaieb, A. Bakrouf, C. Magné, and C. Abdelly, Antioxidant and antimicrobial activities of the edible medicinal halophyte *Tamarix gallica* L. and related polyphenolic constituents. *Food Chem Toxicol.*, **47(8)**, 2083-2091(2009).