KOREAN J ORGANIC AGRI
Volume 21, Number 1: 105-113, March 2013
http://dx.doi.org/10.11625/KJOA.2013.21.1.105

ISSN 1229-3571 (Print)
ISSN 2287-819X (Online)

연구보문

Screening of Antibacterial Activity Against to *Staphylococcus aureus*, *Listeria monocytogens*, *Mannhemia haemolytica* and *Salmonella gallinarum* using Different Plant Extracts

Ham, Young-Joo* · Yang, Jin-Ho** · Na, Chong-Sam***

다양한 식물들을 이용한 *Staphylococcus aureus*, *Listeria monocytogens*, *Mannhemia haemolytica* 및 *Salmonella gallinarum* 항균 추출물 탐색

함영주 · 양진호 · 나종삼

Antibacterial activity is an important feature for the development of antibiotics alternatives. Plant extract is considered as a promising alternative for organic farming. In this study, a total of 11 plants were extracted using ethanol to determine their antibacterial activities against to *Staphylococcus aureus*, Listeria monocytogens, *Mannheimia haemolytica* and *Salmonella gallinarum*. The synergistic interaction among plant extracts was also investigated. Plants used in this study were *Carthamus nctoricus* L. (pA), *Poncirus trifollata* Raf. (pB), *Scutellaria balcalensis* Georgi (pC) *Prunus sargentii* (pD), *Cucurbita moschata* Duch Leaf (pE), *Allium cepa* L. peel (pF) *Portulaca oleracea* L. (pG), *Xanthium strumarium* L. (pH), *Duchesnea chrysantha* (pI), *Cudrania tricuspidata* (pJ) and *Juniperus chinensis* L. (pK). The pB and pA had the most broad antibacterial spectrum and the highest activity against to *Staph. aureus* among plant extract, respectively. In the synergistic interaction, the mixtures of pA and pC as well as pA and pF had batter antibacterial activity against to *Staph. Aureus* compared with other mixtures.

Key words : *antibacterial activity, plant extract, synergistic interaction, Staphylococcus aureus*

* Department of Bioengineering, Konkuk University
** Department of Animal Science, Chonbuk National University
*** Corresponding author, Department of Animal Biotechnology, Chonbuk National University (csna@jbnu.ac.kr)

Ⅰ. Introduction

Antibiotics have played an important role in animal husbandry since the first development of penicillin in early 20th century (Im et al., 2012). It has been used for the treatment and prevention of diseases and used under the purpose of increasing productivity. Antibiotics are known to increase nutrient uptake efficiency by decreasing enteric pathogenic bacteria that harmful to intestine wall, and its mode of action is highly related to the increment of animal productivity (Kayano et al., 2002). Not long ago, this collateral benefits from use of antibiotics became a main purpose of its use in animal husbandry. However, an important adverse effect of antibiotics use such as residence of antibiotics in meat and dairy products and the outbreak of drug resistance pathogenic bacteria has become to cause many social issues and the use of antibiotics for improving animal productivity is banned recently over the world. As a new current concern about antibiotics and animal husbandry, the need for alternative antibiotics that can reduce adverse effect of antibiotics and being essential for organic animal product production has been increased. Among these alternatives, natural plant extract is regarded as a promising agent and their effect on animal productivity has been investigated in numerous studies (Oliveira et al., 2010). Generally, plant extract contains various metabolites showing beneficial to animal productivity such as increasing nutrient digestibility, stabilization of gut microbial balance between enteric pathogen and lactic acid bacteria, and improvement of intestine immune system (Cho et al., 2007). Particularly, antibacterial activity against to pathogenic bacteria is regarded as the most representative feature of plant extract in animal health. The present study prepared ethanol extracts using total 11 different plant, and their antibacterial activities against pathogenic bacteria - *Staphylococcus aureus*, *Listeria monocytogenes*, *Mannhemia haemolytics*, and *Salmonella gallinarum* - were investigated.

Ⅱ. Materials and Methods

1. Plant and preparation of ethanol extract

In this study, a total of 11 plants, *Carthamus nctoricus* L. (pA), *Poncirus trifollata* Raf. (pB), *Scutellaria balcalensis* Georgi (pC) *Prunus sargentii* (pD), *Cucurbita moschata* D_{UCH} Leaf (pE) *Allium cepa* L. peel (pF) *Portulaca oleracea* L. (pG), *Xanthium strumarium* L. (pH), *Duchesnea chrysantha* (pI), *Cudrania tricuspidata* (pJ), and *Juniperus chinensis* L. (pK) were used. All

plants, except pE and pF, were purchased from Kyung-Dong Medicinal Herbs market located in Seoul, Korea. Plants of pE and pF were purchased from local market. Plant samples were finely ground using cutter miller (Philips HR2860, Netherlands). For the preparation of ethanol extraction from plant samples, 10 g of each plant sample was suspended into 100 mL of ethanol and then the extraction was performed for overnight at room temperature. After extraction, the mixture of ethanol and plant was filtered (Whatman No 541) and the filtrate was concentrated to 10 mL using a rotary evaporator (EYELA N-1110S, Japan).

2. Organic solvent extract fractions preparation

The selected plant extract for showing good antibacterial activity was fractionized using different organic solvents. A total of 5 organic solvents were successively applied in the order of ethanol, hexane, chloroform, ethyl acetate and butanol as described in Fig. 1. After extraction using different organic solvents, the used solvent was evaporated and remaining extracts were re-dissolved in ethanol and then used.

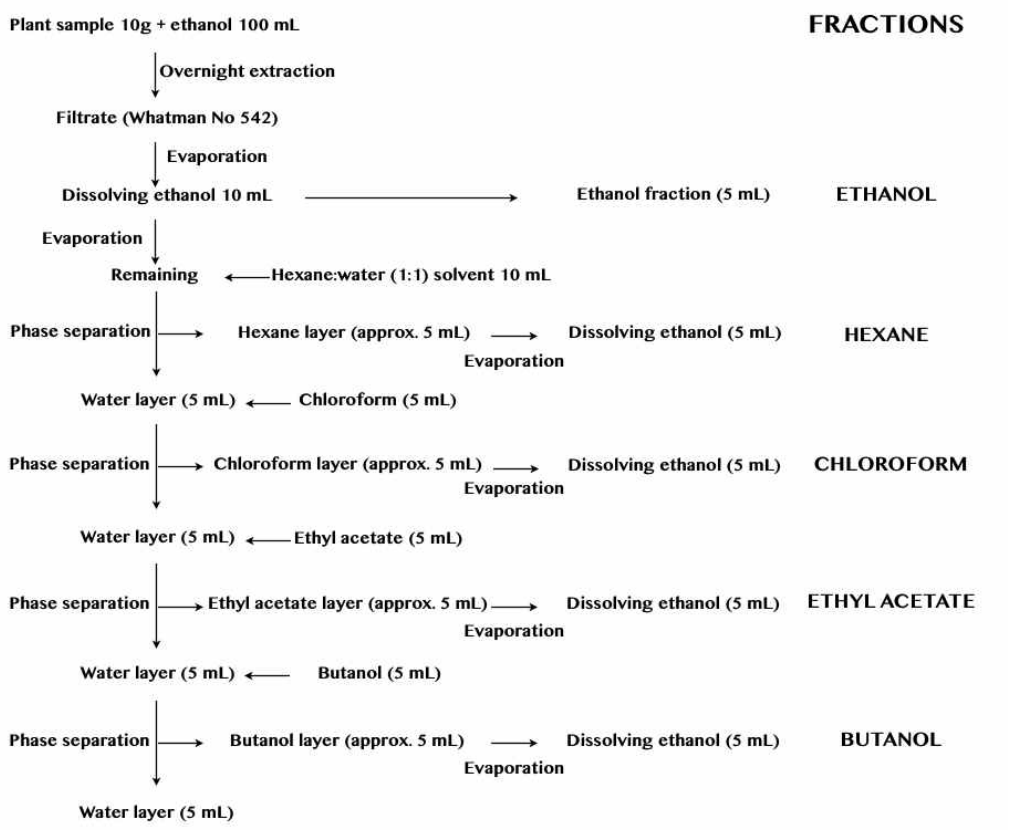

Fig. 1. Schematic diagram for organic solvent fraction preparation

3. Effect of combination among selected plant extracts

To screen the synergistic antibacterial activity among plant extracts and their organic solvent fractions, plant extracts were mixed and their antibacterial activities against to *S. aureus* were investigated.

4. Antibacterial activity

For pathogenic bacteria, *Staph. aureus*, *L. monocytogenes*, *M. heamolytica*, and *Sal. gallinarum* were used. All pathogens were maintained and enriched in LB broth (Difco, USA) for overnight at 37℃. Aliquot 0.1 mL of pathogen culture was added to 10 mL of water agar (0.8% Bacto agar solution), which was previously autoclaved and stored in 50℃ to prevent solidification. The pathogenic suspension in 0.8% agar solution was poured onto LB plate medium and then it was solidified at room temperature. Aliquot 50 uL of a plant extract was added to each paper disc (6 mm in diameter, sterilized), and the paper disc was dried for 30 min to eliminate organic solvent at room temperature. Then the prepared disc was carefully placed on the pathogen culture over-laid water agar plate. Antibacterial activity was determined by measuring appeared diameter of clear zone after overnight incubation of the prepared agar plate at 37℃.

Ⅲ. Results and discussion

In this study, we investigated antibacterial activities against to pathogenic bacteria using ethanol extracts of 11 different plants, *Carthamus nctoricus* L. (pA), *Poncirus trifollata* Raf. (pB), *Scutellaria balcalensis* Georgi (pC) *Prunus sargentii* (pD), *Cucurbita moschata* D_{UCH} Leaf (pE) *Allium cepa* L. peel (pF) *Portulaca oleracea* L. (pG), *Xanthium strumarium* L. (pH), *Duchesnea chrysantha* (pI), *Cudrania tricuspidata* (pJ) and *Juniperus chinensis* L. (pK).

Antibacterial activity of each plant extract was summarized in Table 1. Against to *Staph. aureus*, pA, pB, pC, pE and pF showed antibacterial activities. In diluted extracts, pA, pB and pC retained their activities. Only pB showed antibacterial activity against to *L. monocytogens*. Plant extracts of pB and pF showed antibacterial activity against to *M. haemolytica*. Against to *Sal. gallinarium*, only pB showed antibacterial activity. In the concern of spectrum, pB showed the most broad activity against to all used pathogenic bacteria. For the strength of activity, the largest clear zone diameter was found in pA.

For the investigation of synergistic interaction on antibacterial activity among plant extracts, pA was selected as a fixed factor and remaining plant extracts were mixed with pA in equal volume. And then the mixture of plant extract was loaded on antibacterial activity assay plate containing the culture of *Staph. aureus*. The concentration of individual plant extract concentration was quarter of the first antibacterial activity test. Antibacterial activities of various combinations were shown in Table 2. Good antibacterial activity was found in the combination of pA + pC and pA + pF. Organic solvent fractions of pA, pC and pF were prepared and each plant extract fraction in same organic solvents were mixed and then their antibacterial activities were measured against to *Staph. aureus* (Table 3). For pA + pC, hexane, ethyl acetate and butanol fractions showed higher activity than chloroform fraction. For pA + pF, the highest antibacterial activity was found in hexane fraction.

Table 1. Antibacterial activities of various plant extract against to different pathogenic bacteria

Plants[1]	Pathogenic bacteria															
	Staph. aureus				*L. monocytogens*				*M. hemolytica*				*Sal. gallinarum*			
	Dilution rate				Dilution rate				Dilution rate				Dilution rate			
	1	1/2	1/4	1/8	1	1/2	1/4	1/8	1	1/2	1/4	1/8	1	1/2	1/4	1/8
	Clear zone, mm				Clear zone, mm				Clear zone, mm				Clear zone, mm			
pA	19	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pB	14	13	-	-	13	-	-	-	13	-	-	-	10	-	-	-
pC	10	10	9	-	-	-	-	-	-	-	-	-	-	-	-	-
pD	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pE	10.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pF	13	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-
pG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pJ	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pK	12	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[1] pA, *Carthamus nctoricus* L.; pB, *Poncirus trifollata* Raf.; pC, *Scutellaria balcalensis* Georgi; pD, *Prunus sargentii*; pE, *Cucurbita moschata* D_{UCH} Leaf; pF, *Allium cepa* L. peel; pG, *Portulaca oleracea* L.; pH, *Xanthium strumarium* L.; pI, *Duchesnea chrysantha* (Zoll. & Mor.) Miq.; pJ, *Cudrania tricuspidata*; pK, *Juniperus chinensis* L.

Table 2. Effect of extracts combination on antibacterial activity against to *Staphylococcus aureus*

Mixture	Antibacterial activity, clear zone
pA + ethanol	-
pA + pB	-
pA + pC	++
pA + pD	-
pA + pF	++
pA + pK	+

Clear zone : -, not detected; +, 8~10 mm; ++, 10~12 mm.

Table 3. Antibacterial activities of different organic solvents mixture of *Carthamus nctorius* L. (A), *Scutellaria baicalensis* Georgi (D) and *Allium capa* L. peel (G) against to *Staphylococcus aureus*

Fractions	pA + pC	pA + pF
	Clear zone, mm	
Hexane	14	12
Chloroform	13	11
Ethyl acetate	14	10
Butanol	14	10

Carthamus nctoricus L. is an annual plant involved in Compositae and it is widely cultivated in Egypt, China, Japan and Korea. Its flower and seed are used in oriental medicines and known to have functions of reducing pain, preventing osteoporosis, and treating gynecological bloodstasis (Jang et al., 2012). Serotonin, lignan and flavonoid are known as representative active phenolic compounds in *C. nctoricus* L. (Jang et al., 2012). *Poncirus trifollata* Raf. is a citrus fruit and its common name is trifoliate orange. Generally, citrus fruit is regarded to possess various active compounds such as ascorbic acid, flavanones, phenolics and pectins and known as antioxidant and antibacterial agent (Kim et al., 2012). Skullcap is involved in *Scutellaria baicalensis* Georgi and it is cultivated mainly in Korea, China and Japan. It has been reported to show features of pain killing, anti-inflammation, anti-allergy, antibacterial activity and anti-cancer, and baicalein, wogonin and baicalin were known as major effective compounds in skullcap (Jee et al., 2012).

Prunus sargentii is a tree involved in Rosaceae and it is widely used for afforestation, landscaping and roadside trees. Its bark has been utilized as an oriental medicine for detoxification, the discharge of phlegm, anti-inflammation and the treatment for skin disorders. For active compounds, sakuranin, taxifolin, naringenin and pinostobin were reported and its primary biological functions were antioxidant and anti-bacterial activities (Yang et al., 2012). *Cucurbita moschata* D_{UCH} has unique flavor and it is a favorite vegetable for Korean. It has lots of fatty acids and carotenoids. A compounds related to aglycone has been isolated from *Cucurbita moschata* D_{UCH} and was known to show antibacterial and anti-viral activities (Han et al., 1999). Onion (*Allium cepa* L.) is a perennial plant and widely cultivated over the world. Antioxidant activity derived from quercetin is most representative feature of onion and its anti-bacterial, anti-tumoral and anti-mutagenic activities were also presented (Chang et al., 2010).

Portulaca oleracea L. is a very common plant growing wildly around road and garden. It is used for vegetable food and oriental medicine. As representative compounds, L-noradrenaline, dopamine and dopa were reported, and its effects on arthritis, cancer and cell aging through antioxidant activity were suggested. Recently, antibacterial activity of *P. oleracea* L. extract against to *Helicobacter pylori* was presented (Park et al., 2011). *Xanthium sibiricum* is an annual plant involved in Rosaceae and widely cultivated across Asia, Europe and North America. *X. sibiricum* fruit was reported to have pharmacological features on tumor, pain killing, rhinitis, arthritis, eczema and neuralgia. It was known to show antibacterial activity against to *Staph. aureus*. As active compounds, xanthanol, isoxanthanol, tomentosin, xanthatin, xanthumanol, daucosterol, campesterol and tomesterol were reported (Shin et al., 2012). *Duchesnea chrysanthe* is a perennial plant involved in Rosaceae and has yellow flower and red edible fruit. Ellagic acid, gallic acid, brevifolin carboxylic acid, methyl caffeate, protocatechuric acid and pedunculagin were reported as representative compounds (Lee et al., 2010). *Cudrania tricuspidata* is known as an important oriental medicine for the treatment of cancer in Korea by its antioxidant and anti-inflammatory activities. A compound belong to xanthone family was reported as a representative in *C. tricuspodata* (Lee et al., 2005). *Juniperus chinensis* is an evergreen tree involved in Cupressaceae and distributed particularly in Asia region. Antioxidant, anti-cancer and anti-obesity were reported as health benefit of *J. chinensis*. Cedrol, quercetin, isoquercetin, naringenin, taxifolin, aromadendrin, deoxypodophyllotoxin, catechin, epicatechin, myricitrin and hinokiflavone were presented as biologically active compounds. Ethanol, ethylacetate and aqueous fraction of *J. chinesis* were recently reported to show anti-malarial activity (Lee et al., 2012).

Acknowledgement

This work was supported by “Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ907055)” Rural Development Administration, Republic of Korea.

[논문접수일 : 2013. 1. 19. 논문수정일 : 2013. 2. 25. 최종논문접수일 : 2013. 3. 16.]

Reference

1. Chang, W.-K., S.-B. Cho, D.-W. Kim, S.-S. Lee, and S.-K. Kim. 2010. Cell Growth and Antioxidant Activity on Onion Juice Fermentation by Using *Lactobacillus plantarum* as Animal Probiotics. J. Life Sci. 20: 1729-1737.
2. Cho, J.-I., H.-K. Kang, M.-N. Moon, Y.-B. Lee, and Y.-K. Yang. 2007. Development of bio-formula complex for domestic animal feeding. Korean Journal of Organic Agriculture 15: 93-108.
3. Han, J.-T., E.-M. Ahn, and N.-I. Baek. 1999. Development of biological active compounds from edible plant sources-II. Isolation of fatty acids and sterol glycosides from the leaves of *Cucurbita moschata* DUCH. J. Korean Soc. Agric. Chem. Biotechnol. 42: 267-270.
4. Im, D. Y. and K. I. Lee. 2012. Nitric oxide production inhibitory effect and antibacterial activity of the extract and fractions from Paeoniae Radix. Kor. J. Pharmacogn. 43: 173-178.
5. Jang, S. J. and K. S. Lee. 2012. Determination of antioxidant activity and phenolic compounds in germinated safflower seeds. Journal of Agricultural & Life Science 43: 42-48.
6. Jee, B. Y., J. S. Seo, E. J. Jeon, E. H. Lee, H. J. Choi, J. D. Kim, S. H. Jung, and M. A. Park. 2012. Effects of various concentrations of skullcap extract in the diets on disease resistance of olive flounder Paralichthys olivaceus. J. Fish Pathol. 25: 21-30.
7. Kayano, S.-I., H. Kikuzaki, N. Fukutsuka, T. Mitani, and N. Nakatani. 2002. Antioxidant activity of prune (*Prunus domestica* L.) constituents and a new synergist. J. Agric. Food. Chem. 50: 3708-3712.
8. Kim, S. Y. and K.-S. Shin. 2012. Antimicrobial activity of trifoliate orange (Poncirus trifoliate) seed extracts on Gram-positive foodborne pathogens. Korean J. Food Nutr. 25: 284-290.

9. Lee, B. W., J. H. Lee, S.-T. Lee, H.-S. Lee, W. S. Lee, T.-S. Jeong, and K. H. Park. 2005. Antioxidant and cytotoxic activities of xanthones from *Cudrania tricuspidata*. Bioorganic & Medicinal Chem. Lett. 15: 5548-5552.
10. Lee, K.-S., M.-C. Rho, Y.-J. Jang, J.-E. Lee, A.-J. Choi, C.-B. Park, S.-W. Cha, and D.-J. Lee. 2010. Comparison of biological activities of different parts in *Duchesnea chrysanthe* Miq. Korean J. Intl. Agri. 22: 272-276.
11. Lee, K. H., B. S. Kim, Y. H. Choe, and K. H. Rhee. 2012. In vitro anti-malarial activity of *Juniperus Chinensis* extract. Kor. J. Parmacogn. 43: 239-242.
12. Oliveira, R. A., C. D. Narciso, R. S. Bisinotto, M. C. Perdomo, M. A. Ballou, M. Dreher, and J. E. P. Santos. 2010. Effects of feeding polyphenols from pomegranate extract on health, growth, nutrient digestion, and immunocompetence of calves. J. Dairy Sci. 93: 4280-4291.
13. Park, S.-H., D.-K. Kim, and J.-H. Bae. 2011. The antioxidant effect of *Portulaca oleracea* extracts and its antimicrobial activity on *Helicobacter pylori*. Korean J. Food & Nutr. 24: 306-311.
14. Shin, S. W., J. H. Lee, and B. K. Soo. 2012. Antioxidant and antimicrobial activities of *Xanthium sibiricum*. Korean J. Plant Res. 25: 372-378.
15. Yang, S. A., J. H. Cho, B. S. Pyo, S. M. Kim, and K. I. Lee. 2012. Comparison of the physiological activities of extracts from different parts of *Prunus sargentii*. Korean J. Medicinal Crop Sci. 20: 159-164.