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Antimicrobial activity of the constituents of *Smallanthus sonchifolius* leaves against methicillin-resistant *Staphylococcus aureus*

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Abstract. – *Background and Objectives:* **Methicillin-resistant *Staphylococcus aureus* (MRSA) has been a serious problem as its infection is associated with higher mortality and increase cost worldwide. In the present study, the antibacterial activity of enhydrin, polymatin B, *allo*-schkuhriolide from the leaves of *Smallanthus sonchifolius* was investigated.**

Material and Methods: **Enhydrin, polymatin B, *allo*-schkuhriolide from the leaves of *Smallanthus sonchifolius* were tested for antimicrobial activity using micro dilution broth method against 2 strains of ATCC 33591, ATCC 25923 and 15 strains of clinical isolates MRSA.**

Results: **The antibacterial activity of *Smallanthus sonchifolius* can safely be attributed to enhydrin as polymatin B, and *allo*-schkuhriolide are not showing any activity against *Staphylococcus aureus* strains. The enhydrin showed good antibacterial activity against all tested strains (MIC = 125-500 µg/ml).**

Discussion: **These results suggest that only enhydrin can be considered as an antibacterial drug against MRSA.**

Key Words:

Antibacterial activity, MRSA, *Smallanthus sonchifolius*.

Introduction

Staphylococcus aureus is a public, fatal pathogen associated with a variety of infections and the principal cause of skin and soft tissue infections, surgical site and catheter infections, pneumonia, osteoarticular and bacteremia infections[1,2]. Moreover, because *Staphylococcus aureus* generally is an intracellular pathogenic organ, infections caused by this microbe can be difficult for medical treatment and can survive and relapse. Also, the overuse of drugs causes resistance[1]. Currently, over 50% of the *Staphylococcus aureus* infections around the world are caused by methicillin-resistant *Staphylococcus aureus* (MRSA)[3]. The rise in antibiotic-resistant pathogens has led to the development of new therapeutic agents that are effective against these bacteria. Recently, there has been considerable interest in the use of plant materials as an alternative method to control pathogenic microorganisms[4] and many compounds of plant products have been shown to be specifically targeted against resistant pathogenic bacteria[5].

Yacon [*Smallanthus sonchifolius* H. Robinson; Asteraceae; syn *Polymnia sonchifolia*] is an Andean crop used for centuries by the native inhabitants of South America as food and in traditional medicine[6]. *Smallanthus sonchifolius* is reported to have antioxidative[7], antiinflammatory[8] and antimicrobial[9], activity properties. The constituents of tubers of *Smallanthus sonchifolius* include fructooligosaccharide and phenolic compounds[9,10] and the leaves have several kaurene diterpenoids, acetophenone-type phytoalexins, and melampolide-type sesquiterpene lactones[8]. However, to date, no studies regarding the antimicrobial activity of the leaves of *Smallanthus sonchifolius* against MRSA have been conducted. Therefore, the goal of this study is to evaluate the antimicrobial activity of enhydrin, polymatin B and allo-schkuhriolide from the leaves of *Smallanthus sonchifolius*.

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Materials and Methods

Plant Materials and Sample Preparation

The leaves of yacon (*Smallanthus sonchifolius*) were collected from Bonghwa, Gyeongbuk, Korea in September 2005. The plant material was identified by Emeritus Professor Kyong Soon Lee at Chungbuk National University. A voucher specimen of this plant was deposited at the Herbarium of College of Pharmacy, Chungbuk National University, South Korea. Isolated compounds used were enhydrin, polymatin B, *allo*-schkuhriolide from the leaves of *Smallanthus sonchifolius*[8].

Bacterial Strains and Growth Conditions

Staphylococcus aureus and MRSA strains (Table I) were selected as test microorganisms as for 2 decades, therapeutics options have been very limited. In the case of MRSA, it is resistant not only to β-lactams but to other types of antibiotics[11]. Microorganisms were suspended in Mueller Hinton broth (Becton Dickinson, Sparks, MD, USA) and then incubated at 37°C for 24 h. Mueller Hinton agar (Becton Dickinson, Sparks, MD, USA) was used for the agar diffusion method.

Determination of the MecA Gene

Detection of the mecA gene in the MRSA strains was performed by PCR (Polymerase Chain Reaction) amplication. Prior to the DNA extraction, bacteria stock cultures were subcultured twice onto Mueller Hinton agar plates (MHA plates). For rapid extraction,one to five bacterial colonies were suspended in 300 µl of cell lysis buffer and heated at 100°C for 20 minutes. After centrifugation at 12,000 rpm for 10 minutes, 2 µl of the supernatant was used for the DNA extraction. PCR reactions were performed using a MRSA Primer Mix Kit (Genotek Co, Daejeon, Yuseong-gu, Republic of Korea). The PCR amplification consisted of 30 cycles (94°C, 60 sec; 55°C, 60 sec; 72°C, 60 sec). The primers used in this study were as follows: mecA – forward primer: 5'-ATGAGATTAGGCATCGTTTC-3' reverse primer: 5'-TGGATGACAGTACCTGAGCC-3'. The final PCR products were separated on 2% agarose gel.

Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) was determined using the broth dilution method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[12]. Briefly, a

Table I. The *Staphylococcus aureus* strains used in the experiments.

Staphylococcus aureus strain	Class	Mec A gene	Antibiotic resistance pattern
ATCC25923	MSSA	–	–
ATCC33591	MRSA	+	AM, OX
Clinical isolates			
DPS-1	MRSA	+	AM, OX
DPS-2	MRSA	+	AM, OX
DPS-3	MRSA	+	AM, OX
DPS-4	MRSA	+	AM, OX
DPS-5	MRSA	+	AM, OX
DPS-6	MRSA	+	AM, OX
DPS-7	MRSA	+	AM, OX
DPS-8	MRSA	+	AM, OX
DPS-9	MRSA	+	AM, OX
DPS-10	MRSA	+	AM, OX
DPS-11	MRSA	+	AM, OX
DPS-12	MRSA	+	AM, OX
DPS-13	MRSA	+	AM, OX
DPS-14	MRSA	+	AM, OX
DPS-15	MRSA	+	AM, OX

+ = Positive; – = Negative; AM = Ampicillin; OX = Oxacillin.

preparation of the microorganisms inocula was done on 24 h broth cultures and the suspensions were adjusted to a 0.5 McFarland standard turbidity (approximately 10^8 CFU/ml). Final inoculums were adjusted to the 10^4 CFU/ml. The MHB was supplemented with serial ampicillin and enhydrin, polymatin B, allo-schkuhriolide concentrations. The MIC was defined as the lowest concentration in which there is no visible growth after 24 h of incubation at 37°C[12].

Statistical Analysis

All the experiments were performed in triplicates. The MIC data for each microorganism were analyzed using one-way analysis of variance (ANOVA) and the differences among group means were analyzed using the Dunnett's multiple comparisons test. P value < 0.05 was considered as significant.

Results

Enhydrin, polymatin B, *allo*-schkuhriolide (Figure 1) from the leaves of *Smallanthus sonchifolius* were screen tested for antimicrobial activity using micro dilution broth method against 3 strains of ATCC 33591, ATCC 25923 and DPS-1. The results are presented in Table II as MIC. The bioactivity is only seen in enhydrin compared to the polymatin B, and *allo*-schkuhriolide. Enhydrin appears to be the sole compound showing antibacterial activity with MICs ranging from 125 to 500 µg/ml (Table III).

Discussion

Today, the ongoing emergence of multi-drug resistant bacteria and the infectious diseases caused by them are serious global problems[3]. MRSA is very dangerous, and produces serious medical problems since its infection is often associated with acquired multi-drugs resistance. Today, with this emergence of antibiotic resistant pathogens like MRSA, a new approach using natural products must be taken. These natural products are more and more in demand by their non side effect benefit[13], creating the need to develop alternative antimicrobial drugs for the

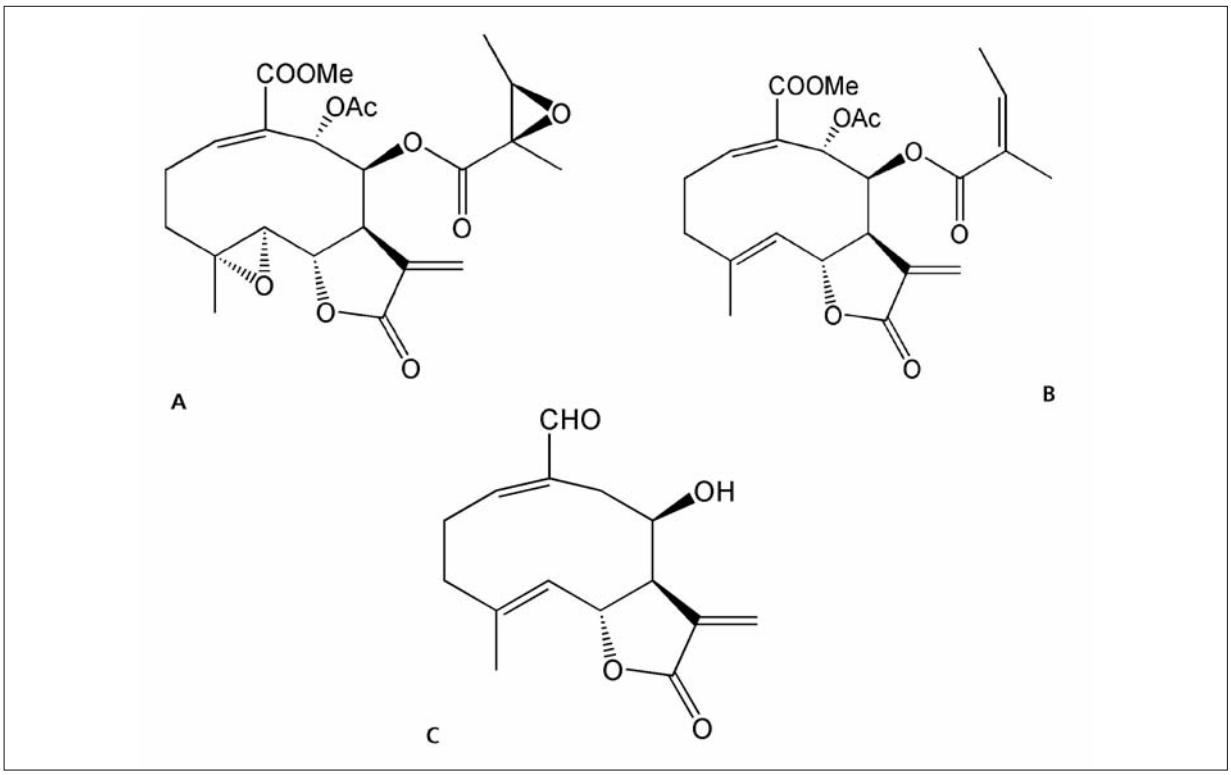

Figure 1. ***A,*** Chemical structure of enhydrin. ***B,*** Polymatin B. ***C,*** *Allo*-schkuhriolide.

Table II. Antimicrobial activity of enhydrin (ED), polymatin B (PM), *allo*-schkuhriolide (AS) from the leaves of *Smallanthus sonchifolius* against *Staphylococcus aureus* (ATCC 33591, ATCC 25923, DPS-1), strains.

Staphylococcus aureus strain	MIC (μg/ml)				
	ED	PM	AS	AM[c]	OX[c]
ATCC25923	250	ND[a]	ND	0.06	0.97
ATCC33591	125	ND	ND	500	500
DPS-1[b]	250	ND	ND	250	250

[a]ND; no detected activity at this concentration; [b]DPS, clinical isolates from Wonkwang University Hospital; [c]Positive control; ampicillin (AM) and oxacillin (OX).

treatment of infectious diseases[14,15]. Thus, our ongoing efforts to find bioactive natural products have led us to study the antibacterial activity of *Smallanthus sonchifolius* against MRSA strains. The antibacterial activity of *Smallanthus sonchifolius* can safely be attributed to enhydrin as polymatin B, and *allo*-schkuhriolide are not showing any activity against *Staphylococcus aureus* strains. The enhydrin showed good antibacterial activity against all tested strains (MIC = 125-500 μg/ml). From the structure of melampolides derivatives isolated from *Smallanthus sonchifolius*, enhydrin like the other two are different in their polymatin B, and *allo*-schkuhriolide enhydrin seems, by its different biological properties, to be an object of extensive research projects to better the health of human kind.

In light of the results obtained, the antibacterial activity of *Smallanthus sonchifolius* can safely be attributed to enhydrin as polymatin B, and *allo*-schkuhriolide are not showing any activity against *Staphylococcus aureus* strains. This com-

Table III. Antimicrobial activity of enhydrin(ED) isolated *Smallanthus sonchifolius* and ampicillin against 16 strains of *Staphylococcus aureus*.

Staphylococcus aureus strain	Class	Mec A gene	MIC[a] (μg/ml)		
			ED	AM[c]	OX[c]
ATCC25923	MSSA	–	250	0.06	0.97
ATCC33591	MRSA	+	125	500	500
Clinical isolates					
DPS-1[b]	MRSA	+	250	250	250
DPS-2	MRSA	+	500	62.5	500
DPS-3	MRSA	+	500	250	500
DPS-4	MRSA	+	500	62.5	250
DPS-5	MRSA	+	250	31.25	250
DPS-6	MRSA	+	250	31.25	500
DPS-7	MRSA	+	250	31.25	500
DPS-8	MRSA	+	250	31.25	500
DPS-9	MRSA	+	250	31.25	250
DPS-10	MRSA	+	500	31.25	500
DPS-11	MRSA	+	500	31.25	500
DPS-12	MRSA	+	500	31.25	500
DPS-13	MRSA	+	250	31.25	500
DPS-14	MRSA	+	500	31.25	500
DPS-15	MRSA	+	250	31.25	500

[a]MIC = Minimum inhibitory concentration; [b]DPS = Clinical isolates from Wonkwang University Hospital; [c]Positive control = Ampicillin (AM) and oxacillin (OX).

pound isolated from *Smallanthus sonchifolius* possessed antimicrobial activity *in vitro* and can be considered as potential candidate drug in the treatment of infectious diseases caused by MRSA. However, a measure of caution should be taken as herbal medicines can be toxic particularly for the liver[16]. Further investigations are therefore on the way regarding the biological activities of enhydrin and its toxicity.

Acknowledgements

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References

1) Baltch AL, Ritz WJ, Bopp LH, Michelsen PB, Smith RP. Antimicrobial activities of daptomycin, vancomycin, and oxacillin in human monocytes and of daptomycin in combination with gentamicin and/or rifampin in human monocytes and in broth against *Staphylococcus aureus*. Antimicrob Agents Chemother 2007; 51: 1559-1562.

2) Denis O, Deplano A, Nonhoff C, Hallin M, De RR, Vanhoof R, De MR, Struelen MJ. *In vitro* activities of ceftobiprole, tigecycline, daptomycin, and 19 other antimicrobials against methicillin-resistant *Staphylococcus aureus* strains from a National Survey of Belgian Hospitals. Antimicrob Agents Chemother 2006; 50: 2680-2685.

3) Mahamat A, MacKenzie FM, Brooker K, Monnet DL, Daures JP, Gould IM. Impact of infection control interventions and antibiotic use on hospital MRSA: a multivariate interrupted time-series analysis. Int J Antimicrob Agents 2007; 30: 169-176.

4) Aqil F, Khan MS, Owais M, Ahmad I. Effect of certain bioactive plant extracts on clinical isolates of beta-lactamase producing methicillin resistant *Staphylococcus aureus*. J Basic Microbiol 2005; 45: 106-114.

5) Nostro A, Cellini L, Di BS, Cannatelli MA, Di CE, Procopio F, Grande R, Marzio L, Alonzo V. Effects of combining extracts (from propolis or Zingiber officinale) with clarithromycin on Helicobacter pylori. Phytother Res 2006; 20: 187-190.

6) Valentova K, Sersen F, Ulrichova J. Radical scavenging and anti-lipoperoxidative activities of *Smallanthus sonchifolius* leaf extracts. J Agric Food Chem 2005; 13: 5577-5582.

7) Yan X, Suzuki M, Ohnishi-Kameyama M, Sada Y, Nakanishi T, Nagata T. Extraction and identification of antioxidants in the roots of yacon (*Smallanthus sonchifolius*). J Agric Food Chem 1999; 47: 4711-4713.

8) Hong SS, Lee SA, Han XH, Lee MH, Hwang JS, Park JS, Oh KW, Han K, Lee MK, Lee H, Kim W, Lee D, Hwang BY. Melampolides from the leaves of *Smallanthus sonchifolius* and their inhibitory activity of lps-induced nitric oxide production. Chem Pharm Bull 2008; 56: 199-202.

9) Lin F, Hasegawa M, Kodama O. Purification and identification of antimicrobial sesquiterpene lactones from yacon (*Smallanthus sonchifolius*) leaves. Biosci Biotechnol Biochem 2003; 67: 2154-2159.

10) Ohyama T, Ito O, Yasuyoshi S, Ikarashi T, Minamisawa K, Kubota M, Tsukihashi T, Asami T. Composition of storage carbohydrate in tubers of yacon (*Polymnia sonchifolia*). Soil Sci Plant Nutr 1990; 36: 167-171.

11) Hu ZQ, Zhao WH, Asano N, Yoda Y, Hara Y, Shimamura T. Epigallocatechin gallate synergistically enhances the activity of carbapenems against methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2002; 46: 558-560.

12) Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standards. CLSI document M7-A5. CLSI; Wayne, PA, USA; 2000.

13) Ghosh A, Das BK, Roy A, Mandal B, Chandra G. Antibacterial activity of some medicinal plant extracts. J Nat Med 2008; 62: 259-262.

14) Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol 1998; 62: 183-193.

15) Choi JG, Kang OH, Lee YS, Oh YC, Chae HS, Jang HJ, Shin DW, Kwon DY. Antibacterial activity of methyl gallate isolated from Galla Rhois or carvacrol combined with nalidixic acid against nalidixic acid resistant bacteria. Molecules 2009; 11: 1773-1780.

16) Valente G, Sanges M, Campione S, Bellevicine C, De Franchis G, Della Gatta R, Sollazzo R, Mattera D, Cimino L, D'arienzo A. Herbal hepatotoxicity: a case of difficult interpretation. Eur Rev Med Pharmacol Sci 2010; 14: (in press).