A African Journal of Pharmacy and Pharmacology Vol. 5(18), pp. 2056-2061, 15 November, 2011
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP11.391
ISSN 1996-0816 © 2011 Academic Journals

Full Length Research Paper

Antibacterial effect of *Euphorbia supina* extracts against methicillin-resistant *Staphylococcus aureus* under dark and light intensity

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Accepted 27 October, 2011

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious clinical urgent problem worldwide. Few new drugs are available against methicillin-resistant Staphylococcus aureus (MRSA), because MRSA has the ability to acquire resistance to most antibiotics, which consequently increases the cost of medication. In the present study, the antibacterial activity of *Euphorbia supina* has been investigated. The antibacterial activities of EtOH extract of *E. supina* and its *n*-hexane, EtOAc, *n*-butanol and water fractions were evaluated against 6 strains of methicillin-resistant *S. aureus* and 1 standard methicillin-susceptible *S. aureus* (MSSA) strain by using the disc diffusion method, minimal inhibitory concentrations (MICs) and colorimetric assay using 3- 4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) test under dark and light intensity from 2,000 to 18,000 lux. Under dark, the *n*-hexane fraction of *E. supina* had a MIC of 31.25 µg/ml. Antimicrobial activity of all the fractions was remarkable especially under illumination from 14,000 to 16,000 lux. *E. supine* can be considered an alternative MRSA treatment.

Key words: *Euphorbia supina,* milk purslane, antibacterial, methicillin-resistant *Staphylococcus aureus* (MRSA), light intensity.

INTRODUCTION

Staphylococcus aureus is a bacterium that grows in the human nose and skin and is a major pathogen for the skin and soft-tissue infections. Methicillin antibiotics have been used against *S. aureus*; however, since its detection in 1961, methicillin-resistant *S. aureus* (MRSA) has become the most problematic Gram-positive bacterium in the public health arena (Witte, 1999). MRSA is an organism that represents a worldwide threat owing its ability to acquire resistance to most antibiotics (Gibbons, 2004; Aqil et al., 2006). This pathogen is associated with a variety of infectious diseases (Baltch et al., 2007) and has an average mortality rate of 36 to 50% (Dancer, 2008). With increasing antimicrobial resistance to various drugs, combination therapy appears to be a

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useful option, particularly in developing countries where the availability of drugs is limited (Aqil et al., 2006; Miranda-Novales et al., 2006). Furthermore, MRSA strains are resistant not only to beta-lactam antibiotics, but also to fluoroquinolones and other families of antibiotics (Aqil et al., 2006). The primary purpose of this study was to investigate the *in vitro* effect of *Euphorbia supina* against MRSA whether there is light intensity or not. We concluded that *E. supina* exhibits considerable antibacterial activity against MRSA in the presence of light and hexane fraction. *E. supina* is an annual herb that grows in the field. Its main stream spreads on the ground and its length is about 10 to 25 cm, containing leaves and red-colored hair. On the center of it, there is red-colored macule with white latex (Lee, 2003). It has been used as a home remedy for hemostasis, diuretic, strong stomach detoxification, smoothening the milk flow (is smooth), activating blood circulation, treating jaundice, diarrhea, dysentery, blood in the urine, excessive uterine bleeding, bleeding due to a wound and carbuncle, the virus of a boil (as a home remedy). As a kind of folk remedy, it has been used (to treat) as antibiotic and antiparasitic for detoxification and hemostasis (zhonghuabencao, 1999). When it comes to constituents of *E. supina*, they have been reported as triterpenoid, derivatives (Tanaka, 1991), monoterpenelactone (Tanaka, 1985), tannins, flavonoids and phenol (Tanaka, 1991). In addition, one type of organism's vitality has been found to be anticancer treatments *in vitro* (Tanaka, 1990). Although, the antibacterial activity of *E. supina* has not been studied, we conducted trials to evaluate its antibacterial activity against MRSA.

MATERIALS AND METHODS

Plant material and sample preparation

E. supina were collected from Sunchon National University, Southern Republic of Korea, in June, 2010. Samples were identified by Professor Dong-Young Shin of the Department of Development in Plant Resources. A voucher specimen was deposited in the Laboratory of Oriental Pharmacology (N.1360). *E. supina* were air-dried to 16 g, which were then boiled in 1 L of ethanol for 3 h. The ethanol extract of *E. supina* (8.63% w/w) was partitioned with organic solvents of different polarities to yield *n*-hexane, EtOAc, *n*-BuOH and water fractions, in sequence. The samples were stored at 4°C.

Equipment

An incubator (Vision, Korea), plant growth chamber (Vision, Korea) and Shinkwang Electric L-40D compact fluorescent Lamp were used.

Test microorganisms

Five clinical isolates (MRSA) were obtained from five different patients at Wonkwang University Hospital (Iksan, South Korea).

The other 2 strains were *S. aureus* ATCC 33591 (Methicillin-resistant strain) and *S. aureus* ATCC 25923 (Methicillin-susceptible strain). Before use, all bacteria were stored in 30% glycerol and frozen at -70°C. The bacteria were cultured in Mueller-Hinton Broth (MHB) and Mueller-Hinton Agar (MHA) (Difco Laboratories, Baltimore, MD, USA). Bacteria were suspended in Mueller-Hinton Broth and then incubated at 37°C for 24 h.

Antibiotics

Ampicillin (AM) and Oxacillin (OX) (Sigma Chemical Co. St.Louis, M0, USA) were used.

Disc diffusion method

The disc diffusion method was (as) described by the Clinical and Laboratory Standards Institute (standards) and by using a modified agar-well diffusion method (CLSI, 2001). Bacterial strains grown on MHA at 37°C for 18 h were suspended in MHB and adjusted to a turbidity of 0.5 McFarland standard scale (approximately 1.5×10^8CFU/ml). The MHA was poured into petri dishes and inoculated with 100 µl of the suspension sterile paper disks (diameter 6 mm: Tokyo Roshi Kaihsa, Japan) were punched in the agar and filled with 500 µg. The dissolution of the organic extracts was facilitated with the addition of 50% (v/v) dimethyl sulfoxide (DMSO) (50% DMSO was not active against all strains) (DMSO, Sigma, USA). AM and OX (was) were used as positive controls, and the discs treated with DMSO were used as the negative control. The plates were placed in a plant growth chamber at 37°C for 18 h under light from 2000 to 18000 lux and dark conditions. The inhibition zone diameter around each of the discs was measured and recorded at the end of the incubation period.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined using the broth microdilution method according to the Clinical and Laboratory Standards Institute Guideline (CLSI, 2000). Briefly, a preparation of the microorganisms inoculated was done on 24 h broth cultures, and the suspensions were adjusted to a 0.5 McFarland standard turbidity (approximately 1.5×10^8 CFU/ml). Final inoculums were adjusted to the 1.5×10^6 CFU/ml. These serially diluted cultures were then incubated at 37°C and exposed to light from 2000 to 18000 lux for 18 h. MIC was defined at the lowest concentration of AM, OX, *E. supina* extracts and fractions (n-Hexane and EtoAc). At the end of the incubation period, the well plates were visually examined for turbidity. Cloudiness indicates that bacterial growth has not been inhibited by the concentration of antimicrobial agent contained in the medium. A colorimetric assay for rapid detection of the presence of bacteria was also performed (Colorimetric assay using 3- 4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide [MTT] test).

Colorimetric assay using MTT test

A colorimetric assay based on MTT for rapid detection of the presence of bacteria was performed as previously described (Scheuber et al., 1983; Abate et al., 1998; Shi et al., 2008). Briefly, a stock solution of 5 mg/ml MTT (Sigma) was prepared in phosphate-buffered saline and was kept at -70°C. A final concentration of 1 mg/ml of MTT was used in the assay. After 24 h

Table 1. Antimicrobial activity of *E. supina* ethanol extract against *S. aureus* strain ATCC 33591 and 25923, DPS 1, DPS 2, DPS 3, DPS 4 and DPS 5 under dark and light intensity.

	Minimal inhibitory concentration (MIC) (µg/ml)						
***S. aureus* strain**	**ATCC 33591**	**ATCC 25923**	**DPS1[a]**	**DPS2**	**DPS3**	**DPS4**	**DPS5**
Dark	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**	**ND**
2000 lux	250	125	250	31.25	125	125	125
4000 lux	125	125	250	31.25	31.25	31.25	31.25
6000 lux	62.5	62.5	62.5	15.625	31.25	31.25	7.8
8000 lux	62.5	62.5	31.25	0.975	31.25	31.25	0.243
10000 lux	62.5	62.5	15.625	0.243	0.243	0.243	0.243
12000 lux	31.25	31.25	15.625	0.243	0.243	0.243	0.243
14000 lux	31.25	15.625	0.243	0.243	0.243	0.243	0.243
16000 lux	15.625	7.8	15.625	0.975	15.625	31.25	0.975
18000 lux	31.25	15.623	0.975	0.975	15.625	31.25	0.975

DPS1[a] indicates *Staphylococcus* strains from the Department of Plastic Surgery, Wonkwang University Hospital; ND indicates no detected activity at this concentration.

of incubation at 37°C, 20 µl of the yellow MTT was added to the 96-well microtiter plate (0.3 ml volume; Nunc) and was incubated for an additional 20 min. The presence of a blue color indicates the presence of bacteria.

RESULTS

Table 1 ethanol extract had a MIC of 15.625 µg/ml against *S. aureus* ATCC 33591 under illumination at 16000 lux. The *n*-hexane fraction of *E. supina* had a MIC of 31.25 µg/ml under dark. Antimicrobial activity of all the fractions was remarkable, especially under illumination from 14000 to 16000 lux (Table 2).

E. supina extract had no activity against *S. aureus* strain under dark condition. On the other hand, *E. supina* extracts showed antimicrobial activity when exposed to light intensity from 2000 to 18000 lux, especially under illumination at 10000 lux. The mean of inhibition zones produced against the tested bacteria ranged 24 mm (Table 3).

Antimicrobial activity of all the fractions was remarkable, especially under illumination at 12000 lux. The *n*-hexane fraction of *E. supina* showed antimicrobial activity inhibition zone 20 mm under dark (Table 4).

DISCUSSION

Due to the recent appearance of MRSA and the "Super Bacteria" showing resistance to multiple antibiotics, the development of new antibiotics is urgently required, which is even tendered as a social issue. The ability of MRSA to acquire resistance to most antibiotics has significantly increased the worldwide mortality caused by MRSA infection (Gibbons, 2004; Dancer, 2008). The most effective method is to develop antibiotics from the natural products without having any toxic or side effects. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. The aim of this research is to identify the fact that the extract and fractions of *E. supina* have different degrees of antimicrobial activity by lux. At 0 lux, fractions of *n*-hexane were shown to have MIC 0.3125 mg/ml against *S. aureus* strain ATCC 33591, but other fractions did not show any antimicrobial activity. However, as lux increases, the degree of antimicrobial activity also increases. Most of the fractions were found to show strong antimicrobial activity from 10,000 to 16,000 lux, reaching the peak at 16,000 lux. However, antimicrobial activity decreases at 18,000 lux. The result of this research states that antimicrobial activity goes up by the degree of light, but it starts decreasing after 16,000 lux where it reaches the peak.

Conclusion

Conclusively, we found that *E. supina* extracts and *n*-hexane fraction have an antibacterial effect on MRSA and MSSA in the presence of light. For future research, more in-depth study should be conducted to identify few things as follows. First, we need to identify which compounds would change in the presence of light and be responsible for the strong activity. Second, we have to investigate the mechanism of this effect in relation to those compounds.

ACKNOWLEDGEMENT

This study was supported by Sunchon National University Research Fund in 2011.

Table 2. Antimicrobial activity of *E. supina* ethanol extract, *n*-hexane, EtOAc, *n*-BuOH and water fractions against *S. aureus* strain ATCC 33591, ATCC 25923, DPS 1, DPS 2, DPS 3, DPS 4 and DPS 5 under dark and light intensity.

***S. aureus* strain**	**Minimal inhibitory concentration (MIC) (µg/ml)**										
	Fraction	**Dark**	**2000 lux**	**4000 lux**	**6000 lux**	**8000 lux**	**10000 lux**	**12000 lux**	**14000 lux**	**16000 lux**	**18000 lux**
	n-hexane	31.25	15.63	15.63	7.8	7.8	7.8	3.9	1.95	1.95	3.9
	EtOAc	1000	31.25	31.25	15.63	15.63	15.63	7.8	3.9	3.9	7.8
ATCC 33591	*n*-BuOH	ND	1000	250	250	125	62.5	62.5	62.5	62.5	125
	H_20	1000	1000	1000	250	250	250	125	62.5	62.5	250
	n-hexane	62.5	15.63	15.63	15.63	7.8	3.9	3.9	1.95	1.95	1.95
	EtOAc	500	15.63	15.63	15.63	15.63	15.63	3.9	3.9	3.9	7.8
ATCC 25923	*n*-BuOH	2000	500	500	250	250	125	125	125	62.5	62.5
	H20	2000	1000	1000	1000	500	500	250	250	125	125
	n-hexane	125	31.25	31.25	31.25	15.63	3.9	3.9	7.8	3.9	3.9
	EtOAc	2000	62.5	62.5	62.5	31.25	15.63	15.63	15.63	15.63	15.63
DPS1[a]	*n*-BuOH	ND	2000	2000	500	250	125	125	125	125	125
	H_20	1000	2000	2000	1000	500	500	250	125	125	500
	n-hexane	62.5	7.8	7.8	7.8	3.9	0.975	0.975	0.488	0.488	0.975
	EtOAc	1000	15.63	15.63	15.63	15.63	3.9	3.9	1.95	1.95	3.9
DPS2	*n*-BuOH	ND	250	250	125	125	62.5	62.5	31.25	62.5	62.5
	H_20	500	1000	1000	1000	500	250	250	125	125	250
	n-hexane	125	31.25	15.63	7.8	3.9	3.9	1.95	1.95	1.95	3.9
	EtOAc	1000	62.5	62.5	15.63	7.8	7.8	7.8	7.8	7.8	7.8
DPS3	*n*-BuOH	ND	1000	1000	500	250	250	125	125	125	125
	H_20	2000	1000	1000	500	250	250	250	250	125	125
	n-hexane	31.25	15.63	15.63	15.63	15.63	3.9	1.95	0.975	0.975	3.9
	EtOAc	1000	31.25	31.25	15.63	15.63	7.8	3.9	3.9	3.9	7.8
DPS4	*n*-BuOH	ND	500	500	125	125	125	31.25	31.25	31.25	125
	H_20	2000	1000	500	125	125	125	125	125	62.5	62.5
	n-hexane	31.25	15.63	15.63	15.63	3.9	0.488	0.488	1.95	0.488	3.9
	EtOAc	1000	31.25	31.25	31.25	7.8	3.9	3.9	3.9	3.9	7.8
DPS5	*n*-BuOH	ND	500	500	250	250	62.5	31.25	31.25	31.25	125
	H_20	1000	2000	500	250	250	62.5	31.25	31.25	31.25	125

DPS1[a] indicates *Staphylococcus* strains from the Department of Plastic Surgery, Wonkwang University Hospital; ND indicates no detected activity at this concentration

Table 3. Antimicrobial activity of *E. supina* ethanol extract against *S. aureus* strain ATCC 33591, ATCC 25923, DPS 1, DPS 2, DPS 3, DPS 4, DPS 5 under dark and light intensity.

***S. aureus* strain**	**Zone of inhibitory (mm) lux**									
	Dark	**2000 lux**	**4000 lux**	**6000 lux**	**8000 lux**	**10000 lux**	**12000 lux**	**14000 lux**	**16000 lux**	**18000 lux**
	500 µg	**500 µg**	**500 µg**	**500 µg**	**500 µg**	**500 µg**	**500 µg**	**500 µg**	**500 µg**	**500 µg**
ATCC33591	ND	11	10	12	19	24	21	21	19	15

Table 3. Contd.

ATCC25923	ND	10	10	11	17	24	22	20	16	14
DPS1[a]	ND	8	9	10	14	18	20	22	14	14
DPS2	8	14	17	19	21	23	21	20	15	21
DPS3	ND	9	9	9	12	16	20	19	26	15
DPS4	ND	9	12	14	19	22	21	20	24	18
DPS5	ND	11	10	8	15	20	20	20	15	16

DPS1[a] indicates *staphyloccocus* strains from the Department of Plastic Surgery, Wonkwang University Hospital; ND indicates no detected activity at this concentration.

Table 4. Antimicrobial activity of *E. supina* ethanol extract and *n*-hexane, EtOAc, *n*-BuOH and water fractions against *S. aureus* strain ATCC 33591, ATCC 25923, DPS 1, DPS 2, DPS 3, DPS 4 and DPS 5 under dark and light intensity.

***S. aureus* strain**	**Fractions**	**Zone of inhibitory (mm)**									
		Dark	**2000**	**4000**	**6000**	**8000**	**10000**	**12000**	**14000**	**16000**	**18000**
		500 µg	**500 µg**	**500 µg**	**500 µg**	**500 µg**	**500 µg**	**500 µg**	**500 µg**	**500 µg**	**500 µg**
ATCC 33591	*n*-hexane	20	14	12	11	18	23	25	20	2	22
	EtOAc	9	15	15	16	20	23	24	30	24	25
	n-BuOH	ND	ND	ND	ND	ND	20	21	15	20	18
	H_20	ND	ND	ND	ND	ND	ND	18	20	17	14
ATCC 25923	*n*-hexane	14	12	12	12	16	20	24	20	19	17
	EtOAc	ND	13	13	14	18	22	23	20	24	20
	n-BuOH	ND	ND	ND	ND	ND	14	17	13	15	13
	H20	ND	ND	ND	ND	ND	15	18	15	10	10
DPS1[a]	*n*-hexane	19	10	12	10	18	22	23	13	16	17
	EtOAc	9	14	12	12	20	28	24	18	19	19
	n-BuOH	ND	ND	ND	ND	ND	15	17	10	11	14
	H_20	ND	ND	ND	ND	ND	20	22	13	11	10
DPS 2	*n*-hexane	19	16	15	18	20	22	22	18	20	25
	EtOAc	ND	17	17	18	25	30	31	21	25	28
	n-BuOH	ND	ND	ND	9	16	20	24	13	20	28
	H_20	ND	ND	ND	ND	14	18	20	14	16	22
DPS 3	*n*-hexane	16	14	12	11	18	22	24	19	18	20
	EtOAc	ND	15	15	15	21	25	27	24	25	25
	n-BuOH	ND	ND	ND	ND	14	19	22	20	14	18
	H_20	ND	ND	ND	ND	15	19	24	20	22	20
DPS 4	*n*-hexane	13	14	12	12	17	21	22	14	18	16
	EtOAc	ND	11	13	16	22	28	27	23	24	23
	n-BuOH	ND	ND	ND	ND	14	18	20	11	15	17
	H_20	ND	ND	ND	ND	16	20	23	20	18	18
DPS 5	*n*-hexane	18	14	11	11	19	23	22	18	24	20
	EtOAc	10	15	16	16	22	26	22	21	24	21
	n-BuOH	ND	ND	ND	ND	16	20	21	20	20	20

Table 4. Contd.

	H_20	ND	ND	ND	ND	11	15	18	16	20	20

DPS1[a] indicates *Staphylococcus* strains from the Department of Plastic Surgery, Wonkwang University Hospital; ND indicates no detected activity at this concentration

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