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Antimicrobial Activity against Food-hazardous Microorganisms, Dermatophytes, and Pytopathogens and Antioxidative Activity of Sancho Oil

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ABSTRACT

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Background: Although Sancho (*Zanthoxylum schinifolium* Siebold & Zucc) oil has traditionally been used for its antibiotics properties, there is currently a lack of scientific evidence regarding its biological activities. In this study, we investigated the antimicrobial and antioxidant activities of Sancho oil against food-hazardous microorganisms, phytopathogens, and dermatophytes.

Methods and Results: We investigated the antimicrobial activity of Sancho oil against 11 food-hazardous microorganisms, nine phytopathogens, and six dermatophytes. The Sancho oil was found to show the strongest antibacterial activity against *Shigella flexneri* and *Listeria* spp. Sancho oil also showed high antifungal activity against plant pathogens, particularly *Fusarium oxysporum*, and showed antimicrobial activity against dermatophytes such as *Trichophyton rubrum*, *Microsporium canis* and *Candida albicans*. The antioxidant activity of Sancho oil was measured using the DPPH method, and was found to be stronger than that of unrefined oil. Moreover, this activity increased with increasing oil concentration.

Conclusions: We found that Sancho oil showed differing antimicrobial activities against food-hazardous microorganisms, dermatophytes, and plant pathogens. The antimicrobial activity spectrum of Sancho oil was not broad and varied among microbial strains. On the basis of our findings, we consider that Sancho oil could be used an antibacterial material for food-borne *S. flexneri* and *Listeria* spp., a biopesticide for *Fusarium* spp., and a treatment for dermatophytes such as *T. rubrum*.

Key Words: Sancho Oil, Food-hazardous Microorganisms, Dermatophytes, Phytopathogenic Microorganisms

INTRODUCTION

Sancho (*Zanthoxylum schinifolium* Siebold & Zucc) is a deciduous shrub belonging to the Rutaceae. It grows in the

mid-mountain and valley and is 3 - 5 m high. Young leaves and berries are used as spices because of their unique fragrance and acidity, and have long been used in the private sector as traditional spices and medicinal plants (Lee *et al.*, 2003).

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Sancho oil contains limonene, citronellal, phellandrene, sanshool, and flavonoid (Kim *et al.*, 2000). It has been widely used as an anti-inflammatory, diuretic, detoxifying, antiparasitic, anal pain, and dry place. It stimulates the stomach to improve metabolism, helps digestion and stops diarrhea (Kim *et al.*, 2000). Seo *et al.* (2012) also found that oral administration of 200 mg/kg/day *Z. schinifolium* seed oil for 13 weeks improved most immune-related cells and was biologically safe. *Z. schinifolium* oil has been reported to exhibit pesticidal toxicity against *Dermatophagoides farinae*, *D. pteronyssinus* and *Tyrophagus putrescentiae*, and aphids (Lee, 2016).

On the other hand, the plant field has been extensively studied in materials having antioxidant and antimicrobial effects during the development of new materials (Goñi *et al.*, 2009). In particular, various side effects due to allergies have been reported in the case of long-term administration of a chemical synthetic preparation (Lee and Park, 2011). As an alternative, many studies have been conducted on herbal medicines such as plants and traditional medicines.

Sancho oil is very promising antimicrobials and are expected to be developed as a promising species (Lee *et al.*, 2012). Diao *et al.* (2013) reported that essential oils from *Z. schinifolium* has antimicrobial activity against *Staphylococcus epidermidis* and antiviral activity against food-borne viral surrogates, feline calicivirus-F9 (FCV-F9). However, most of the research has been conducted on the biological activities of *Z. schinifolium* essential oils and extracts extracted from stems and fruits with organic solvents (Kim *et al.*, 2000). It is also widely used in *Z. piperitum*, a similar species of *Z. schinifolium*. Therefore, research on *Z. schinifolium* seed oil, Sancho oil, is rare.

In this study, we investigated the antimicrobial and antioxidant activities of various microorganisms such as food-hazardous microorganisms, dermatophytes, and plant pathogens.

MATERIALS AND METHODS

1. Materials

The Sancho (*Zanthoxylum schinifolium* Siebold & Zucc) oil used for the antimicrobial test according to acidity was collected from Hadong J. Farm (Hadong, Korea) on November 25, 2012, and Sancho oil stored for 1 year was used high acidity Sancho oil (HA1), low acidity Sancho oil (LA1) was harvested in November 2013 and examined for its antibacterial activity. Also, all reagents used in the experiment were used as extra pure grade.

The microorganisms used in this study were 11 food-hazardous microorganisms, 6 dermatophytes, and 9 plant pathogens (Table 1). Microbial strains were purchased from KCTC (Korean Collection for Type Cultures, Jeongup, Korea), KCCM (Korean Culture Center of Microorganisms, Seoul, Korea) and KACC (Korean Agricultural Culture Collection, Wanju, Korea). The microorganisms were cultured by inoculating 100 ml of the microbial stock suspension in 5 ml nutrient salt (Difco Laboratories Inc., Detroit, MI, USA) in a shaking incubator at 37°C for 24 hours under aerobic conditions.

2. Antimicrobial activity of Sancho oil against food-hazardous bacteria

Antimicrobial activity assay was determined by the disk agar plate diffusion method. Each test strain was incubated in the Tryptic soy (TS) agar liquid medium for 24 hours, and the plate medium was prepared by coagulating 20 ml of 1.5% agar sterilized in each growth medium in petridish and adding 0.1 ml of each test microorganism. The glass rods were applied to spread evenly on the medium. A paper disc (8 mm, Toyo Roshi Kaicha Ltd., Tokyo, Japan) in which Sancho oil was injected at a certain concentration was adsorbed onto a plate medium, and

Table 1. Microorganisms used in the investigation of antimicrobial activity against food-hazardous microorganisms, dermatophytes, and phytopathogenic microorganisms.

Microorganisms	Strains
Food-hazardous bacteria	<i>Escherichia coli</i> (KCTC1682), <i>Salmonella enterica</i> subsp. <i>enterica</i> (KCTC 12456), <i>Salmonella bongori</i> (KCTC12397), <i>Shigella flexneri</i> (KCTC 2517), <i>Shigella sonnei</i> (KCTC2518), <i>Enterobacter sakazakii</i> (KCTC 2949), <i>Pseudomonas aeruginosa</i> (KCTC1750), <i>Pseudomonas fluorescens</i> (KCTC 12028), <i>Listeria monocytogenes</i> (KCTC3569), <i>Bacillus cereus</i> (KCTC 1012), <i>Staphylococcus aureus</i> subsp. <i>aureus</i> (KCTC1916)
Dermatophytes	<i>Trichophyton mentagrophytes</i> (KCTC6077), <i>Trichophyton rubrum</i> (KCTC 6345), <i>Trichophyton ajelloi</i> (KCCM60451), <i>Microsporium audouinii</i> (KCTC 6346), <i>Microsporium canis</i> (KCTC6349), <i>Candida albicans</i> (KCTC7270)
Phytopathogens	<i>Rhizopus microsporus</i> var. <i>Oligosporus</i> (KACC41363), <i>Aspergillus awamori</i> (KACC41844), <i>Aspergillus niger</i> (KACC40280), <i>Aspergillus oryzae</i> (KCCM60166), <i>Aspergillus sojae</i> (KCCM60354), <i>Penicillium roqueforti</i> (KACC41354), <i>Aspergillus parasiticus</i> (KACC41862), <i>Aspergillus flavus</i> var. <i>Flavus</i> (KCTC6143), <i>Fusarium oxysporum</i> (KACC41083)

30 μl of sterilized water was injected. The antimicrobial effect was compared by measuring the diameter (mm) of inhibition clear zone.

The antibacterial activity according to the acidity of Sancho oil was carried out by the method mentioned above.

3. Antifungal activity test of Sancho oil against phytopathogens

The antifungal activity test of phytopathogens of Sancho oil was done with a slight modification of the method of Mohana and Raveesha (2007). 50 ml potato dextrose agar (PDA) liquid medium was added to a 250 ml Erlenmeyer conical flask, and Sancho oil dissolved in 20 mg/ml dimethyl sulfoxide (DMSO) was added. The flasks were inoculated with 5 mm diameter mycelia disc of phytopathogens taken from 7 days old culture and incubated for 7 days at $22 \pm 1^\circ\text{C}$ under alternate cycles 12 hours. light and 12 hours. darkness. After incubation the content of the each flask was poured into a preweighed Whatman No. 1 filter paper. The filter paper with the mycelial mat was dried in an oven at 60°C until a constant weight was reached. The dry weight of the mycelia was determined by subtracting the weight of the filter paper from the total weight of the filter paper with mycelia. Three replicates were maintained for each treatment. The percent inhibition of mycelial growth was calculated using the formula:

$$\text{Percent inhibition} = C - [T/C \times 100]$$

where C = Mycelial weight in control, T = Mycelial weight in treatment.

The antimicrobial activity test according to the degree of acidity of Sancho oil was carried out as described above.

4. Antifungal activity test of Sancho oil against dermatophytes

Sancho oil was added to sabourud dextrose agar (SDA, Merck KGaA, Darmstadt, Germany) medium and Sancho oil dissolved in 20 mg/ml DMSO. Then, 5 mm diameter dermatophytes discs were inoculated for 7 days and incubated for 12 hours at $22 \pm 1^\circ\text{C}$ for 7 days. The culture conditions were performed in the same manner as the phytopathogens mentioned above. The contents of each flask were pre-weighed by filter paper (Whatman No. 1, Whatman Inc., Florham Park, NJ, USA) poured into filter paper. The filter paper containing mycelium was dried in an oven at 60°C until a constant weight

was reached. The dry weight of the mycelium was measured by subtracting the weight of the filter paper from the total weight of the filter paper using the mycelium. Three replicates were maintained for each treatment. Mycelial growth inhibition was calculated using the same formula as phytopathogens.

5. Measurement of antioxidative activity by DPPH method

In this experiment, a Sancho oil sample (90 - 500 mg/l) and the solution of 0.4 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) dissolved in ethanol were taken and mixed well. After incubation at 20°C for 20 minutes, absorbance was measured at 517 nm. Electron donating ability (EDA, %) was measured by the difference of the absorbance of the sample added and blank sample as a percentage (%).

$$\text{EDA (\%)} = \left[1 - \frac{\text{absorbance value of sample}}{\text{absorbance value of blank sample}} \right] \times 100$$

The antioxidant activity of the purified Sancho oil was investigated. First, refining of Sancho oil was carried out by the method of Kang *et al.* (2017). In Sancho oil purification, Sancho oil was first slowly stirred at a very low speed in a water bath using phosphoric acid and acetic acid at 0.5, 1.0 and 2.0% concentration. The mixture was heated at 40°C - 50°C and maintained at a final temperature of 85°C for 15 - 20 minutes. The mixture was allowed to stand for 20 minutes and the precipitate was removed. The mixture was centrifuged at 2,000 rpm for 30 minutes to remove degumming oil. The antioxidative activity of DPPH method was measured by using the above-prepared Sancho oil and sesame oil.

6. Statistical analysis

All experiments were performed 3 times. Data are presented as means and standard deviation and analyzed by One-way ANOVA using the IBM SPSS statistical package (Ver. 24, IBM Co., Armonk, NY, USA). Means were compared at 5% significance level using Duncan's Multiple Range Test (DMRT) comparison ($p < 0.05$).

RESULTS

1. Antimicrobial activity against food-hazardous bacteria

The antimicrobial activity of Sancho oil against food-hazardous bacteria is shown in Table 2. The antimicrobial activity of Sancho oil against food-hazardous bacteria was

different for each microbial strain. Sancho oil showed strong antimicrobial activity against *S. flexneri* among tested food-hazardous bacteria. In addition, Sancho oil showed also strong antimicrobial activity against *L. monocytogenes* and *E. coil*.

The antimicrobial activity of food-hazardous bacteria was different according to the degree of rancidity of Sancho oil (Table 2). LA1 Sancho oil had lower antibacterial activity than HA1 Sancho oil with higher acid value.

Table 2. Antibacterial activity of Sancho oil according to acidity and microorganisms.

Food-hazardous bacteria	Antibacterial activity by Sancho oil concentration (mm)	
	HA1 ¹⁾	LA1 ²⁾
<i>Escherichia coil</i>	40.87±3.16 ^{abc*}	9.63±8.86 ^a
<i>Salmonella enterica</i>	19.07±1.81 ^f	4.50±4.31 ^{ab}
<i>Salmonella subsp. enterica</i>	39.20±2.69 ^{bcd}	9.27±7.45 ^{ab}
<i>Salmonella bongori</i>	10.20±1.21 ^g	2.40±1.15 ^{ab}
<i>Shigella flexneri</i>	47.57±5.25 ^a	11.23±1.84 ^a
<i>Shigella sonnei</i>	40.03±2.89 ^{abc}	9.43±8.10 ^a
<i>Enterobacter sakazakii</i>	31.97±1.11 ^{de}	7.53±3.35 ^{ab}
<i>Pseudomonas aeruginosa</i>	19.07±1.81 ^f	4.50±0.46 ^{ab}
<i>Pseudomonas fluorescens</i>	34.13±1.46 ^{cde}	8.07±4.41 ^{ab}
<i>Listeria monocytogenes</i>	42.47±3.79 ^{ab}	10.00±1.11 ^a
<i>Bacillus cereus</i>	31.03±9.51 ^e	7.30±2.95 ^{ab}

¹⁾HA1; high acidity Sancho oil, ²⁾LA1; low acidity Sancho oil. Data represent the means ± S.E of three replicates. Different letter for each treatment shows significant difference using Duncan's Multiple Range Test (DMRT) comparison ($p < 0.05$).

2. Antifungal activity against phytopathogens

The antifungal activity of Sancho oil against phytopathogens was investigated (Table 3). Antifungal activity against phytopathogens varied with treatment concentrations. As the concentration of Sancho oil increased, antifungal activity increased in most phytopathogens strains. The antifungal activity of low concentration Sancho oil of less than 3% was highest in *F. oxysporum*, followed by *P. roqueforti*. *F. oxysporum* was the strongest at 5% treatment, followed by *A. awamori*, *A. niger*, *P. roqueforti*, and *A. flavus* var. *flavus*.

The antifungal activity of phytopathogens according to the acidity of Sancho was measured (Fig. 1). The antifungal activities of phytopathogens except *Rhizopus* spp. showed higher antifungal activity in Sancho oil, which has lower acidity than that of high acidity Sancho oil. The antifungal activity of low concentration Sancho oil of less than 3% was highest in *F. oxysporum*, followed by *P. roqueforti*. *F. oxysporum* was the strongest at 5% treatment. *A. awamori* increased sharply from 3% and showed 98% antifungal activity at 6%. The antifungal activity of *A. niger* and *A. flavus* was weaker than *A. awamori*, but showed the same tendency.

3. Antimicrobial activity against dermatophytes

The antimicrobial activity of Sancho oil against six dermatophytes was investigated (Fig. 2). In the growth of dermatophytes, the higher the concentration of Sancho oil, the more the growth of the dermatophytes was inhibited than the control group. Among them, Sancho oil showed strong antimicrobial activity against *M. canis* and *C. albicans*. *T.*

Table 3. Antifungal activity of Sancho oil against phytopathogenic fungi.

Microorganisms	Antifungal activity by Sancho oil oil concentration (%)						
	0	1	2	3	4	5	6
Rm ¹⁾	0.00±0.00 ^{u*}	2.57±3.04 ^{tu}	2.57±3.04 ^{tu}	0.00±0.00 ^u	0.00±0.00 ^u	0.00±0.00 ^u	0.89±0.05 ^{tu}
Aa ²⁾	0.00±0.00 ^u	0.00±0.00 ^u	4.56±3.53 ^{stu}	20.56±0.03 ^{no}	65.37±1.62 ^{gh}	91.58±0.39 ^{cd}	98.13±0.09 ^{ab}
An ³⁾	0.00±0.00 ^u	2.65±0.75 ^{tu}	8.91±0.42 ^{qrs}	30.35±0.16 ^{kl}	59.74±2.16 ⁱ	82.14±0.06 ^e	91.95±0.43 ^{cd}
Ao ⁴⁾	0.00±0.00 ^u	1.36±2.35 ^{tu}	2.57±0.13 ^{tu}	11.10±0.29 ^{pq}	28.08±3.69 ^{lm}	46.92±2.73 ^j	70.03±1.54 ^f
As ⁵⁾	0.00±0.00 ^u	3.04±0.15 ^{tu}	5.90±4.76 st	10.96±4.50 ^{pq}	14.00±4.35 ^p	31.20±3.48 ^{kl}	81.82±0.09 ^e
Pr ⁶⁾	0.00±0.00 ^u	12.38±0.83 ^{pq}	29.52±0.67 ^{kl}	68.58±1.61 ^{fg}	80.96±0.77 ^e	87.62±0.83 ^d	90.48±0.86 ^{cd}
Ap ⁷⁾	0.00±0.00 ^u	0.33±0.57 ^u	9.99±0.9 ^{pqr}	20.02±3.20 ^o	34.02±2.34 ^k	63.01±1.63 ^{hi}	78.00±0.22 ^e
Af ⁸⁾	0.00±0.00 ^u	2.35±2.65 ^{tu}	8.19±0.37 ^{qrs}	24.55±0.21 ^{mn}	62.68±1.70 ^{hi}	79.06±0.95 ^e	88.17±0.54 ^d
Fo ⁹⁾	0.00±0.00 ^u	65.00±0.29 ^{gh}	95.00±0.04 ^{bc}	97.50±0.02 ^{ab}	99.17±0.01 ^{ab}	100.00±0.00 ^a	100.00±0.00 ^a

¹⁾Rm; *Rhizopus microsporus* var. *oligosporus*, ²⁾Aa; *Aspergillus awamori*, ³⁾An; *Aspergillus niger*, ⁴⁾Ao; *Aspergillus oryzae*, ⁵⁾As; *Aspergillus sojae*, ⁶⁾Pr; *Penicillium roqueforti*, ⁷⁾Ap; *Aspergillus parasiticus*, ⁸⁾Af; *Aspergillus flavus* var. *flavus*, and ⁹⁾Fo; *Fusarium oxysporum*. Different letter for each treatment shows significant difference using Duncan's Multiple Range Test (DMRT) comparison ($p < 0.05$).

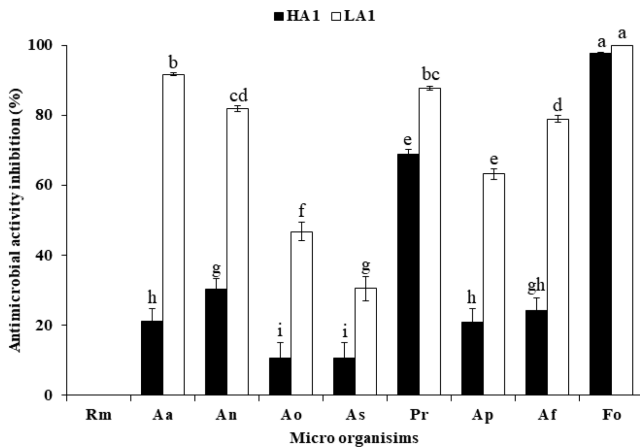


Fig. 1. Antimicrobial activity of Sancho oil against plant pathogenic fungi according to acidity. Rm; *Rhizopus microsporus* var. *oligosporus*, Aa; *Aspergillus awamori*, An; *Aspergillus niger*, Ao; *Aspergillus oryzae*, As; *Aspergillus sojae*, Pr; *Penicillium roqueforti*, Ap; *Aspergillus parasiticus*, Af; *Aspergillus flavus* var. *flavus*, Fo; *Fusarium oxysporum*.

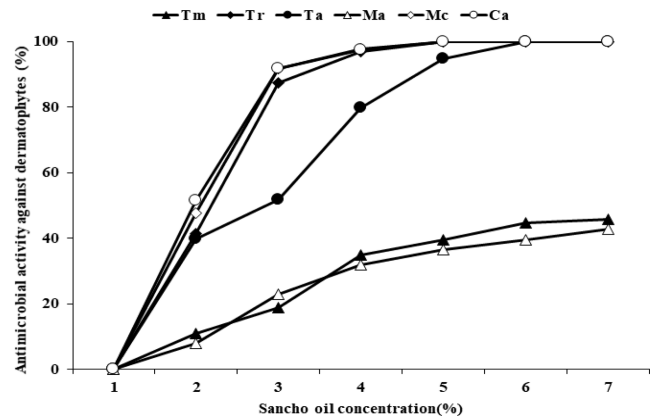


Fig. 2. Antibacterial activity against dermatophytes according to the concentration of Sancho oil. Tm; *Trichophyton mentagrophytes*, Tr; *Trichophyton rubrum*, Ta; *Trichophyton ajelloi*, Ma; *Microsporum audouinii*, Mc; *Microsporum canis*, Ca; *Candida albicans*.

rubrum, *M. canis*, and *C. albicans* did not grow when treated with more than 4% of Sancho oil. However, Sancho oil had low antimicrobial activity against *T. mentagrophytes* and *M. audouinii*.

The antimicrobial activity of Sancho oil against dermatophytes was different according to treatment period (Table 4). Except for *T. mentagrophytes* and *M. audouinii*, it strongly inhibited the growth of dermatophytes from the 2nd day of cultivation. After 4 days, the growth was completely inhibited.

The effect of antimicrobial activity of dermatophytes was shown to be high when Sancho oil with low acid value was used (Fig. 3). However, the growth of *T. mentagrophytes* and *M. audouinii* was lower than that of the control group, but there was no decrease in the acidity of Sancho oil.

4. Antioxidant activity test of Sancho oil

The electron donating ability (EDA, %) values of Sancho oil varied slightly depending on the acidity and concentration of oil (Fig. 4). The EDA value of Sancho oil increased with increasing oil concentration. In the case of HA1, the EDA value gradually increased according to the concentration, while the EDA value rapidly increased in the LA1 up to 90 mg/l, but did not increase significantly at the higher concentration.

Purification yield of phosphate and acetic acid was higher in phosphate treatment than acetic acid treatment (data not shown). Purification yields for these two treatments were 84% - 92%.

The EDA showed the difference of antioxidant ability of Sancho oil purified by phosphoric acid and acetic acid treatment (Fig. 5). The antioxidant ability of oil purified by phosphoric acid treatment was slightly improved compared

Table 4. Antimicrobial activity against dermatophytes with treatment period of Sancho oil.

Dermatophytes	Treatment period of Sancho oil (day)						
	0	1	2	3	4	5	6
Tm ¹⁾	0.00±0.00 ^{m*}	10.89±4.46 ^l	18.84±4.06 ^k	34.91±3.25 ^{ij}	39.58±3.02 ^{ghi}	44.59±2.77 ^{fg}	45.84±2.71 ^{ef}
Tr ²⁾	0.00±0.00 ^m	41.31±2.93 ^{ghi}	87.46±0.63 ^c	96.90±0.16 ^{ab}	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
Ta ³⁾	0.00±0.00 ^m	39.87±3.01 ^{ghi}	51.89±2.41 ^e	79.85±1.01 ^d	94.85±0.26 ^{ab}	100.00±0.00 ^a	100.00±0.00 ^a
Ma ⁴⁾	0.00±0.00 ^m	7.89±4.61 ^l	22.84±3.86 ^k	31.91±3.40 ^j	36.58±3.17 ^{hij}	39.59±3.02 ^{ghi}	42.84±2.86 ^{gh}
Mc ⁵⁾	0.00±0.00 ^m	47.58±2.62 ^{ef}	91.81±0.41 ^{bc}	97.84±0.11 ^{ab}	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a
Ca ⁶⁾	0.00±0.00 ^m	51.58±2.42 ^e	91.81±0.41 ^{bc}	97.65±0.12 ^{ab}	100.00±0.00 ^a	100.00±0.00 ^a	100.00±0.00 ^a

¹⁾Tm; *Trichophyton mentagrophytes*, ²⁾Tr; *Trichophyton rubrum*, ³⁾Ta; *Trichophyton ajelloi*, ⁴⁾Ma; *Microsporum audouinii*, ⁵⁾Mc; *Microsporum canis*, and ⁶⁾Ca; *Candida albicans*. *Different letter for each treatment shows significant difference using Duncan's Multiple Range Test (DMRT) comparison ($p < 0.05$).

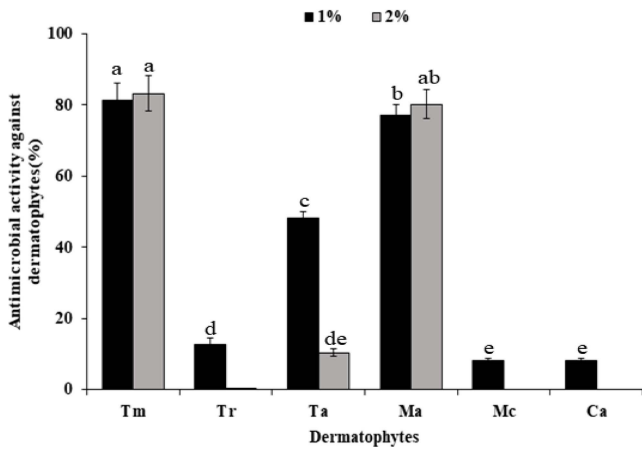


Fig. 3. Antimicrobial activity of Sancho oil with low acidity on dermatophytes. Tm; *Trichophyton mentagrophytes*, Tr; *Trichophyton rubrum*, Ta; *Trichophyton ajelloi*, Ma; *Microsporum audouinii*, Mc; *Microsporum canis*, Ca; *Candida albicans*.

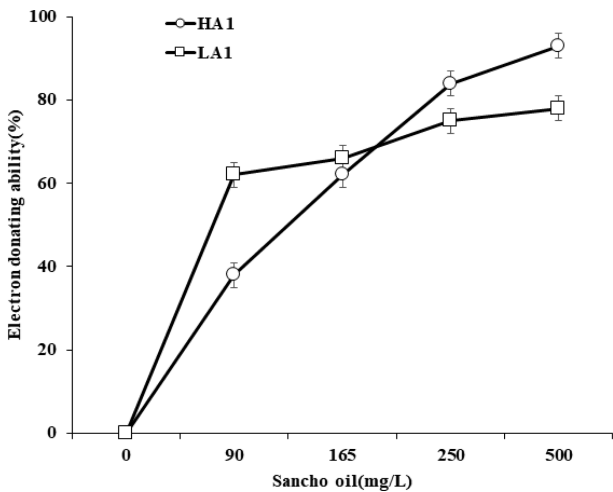


Fig. 4. The free radical scavenging effect of using DPPH method of different Sancho oil. HA1; high acidity Sancho oil, LA1; low acidity Sancho oil.

with that of acetic acid treated oil. The antioxidant ability was slightly increased as the concentration of the two refining reagents was increased. However, there was no significant difference in antioxidant capacity between high and low oil.

DISCUSSION

The antimicrobial activity of Sancho oil against food-hazardous bacteria differed from microorganism to microorganism, among which it was the strongest against *L. monocytogenes*. Lee *et al.* (2012) examined the antimicrobial

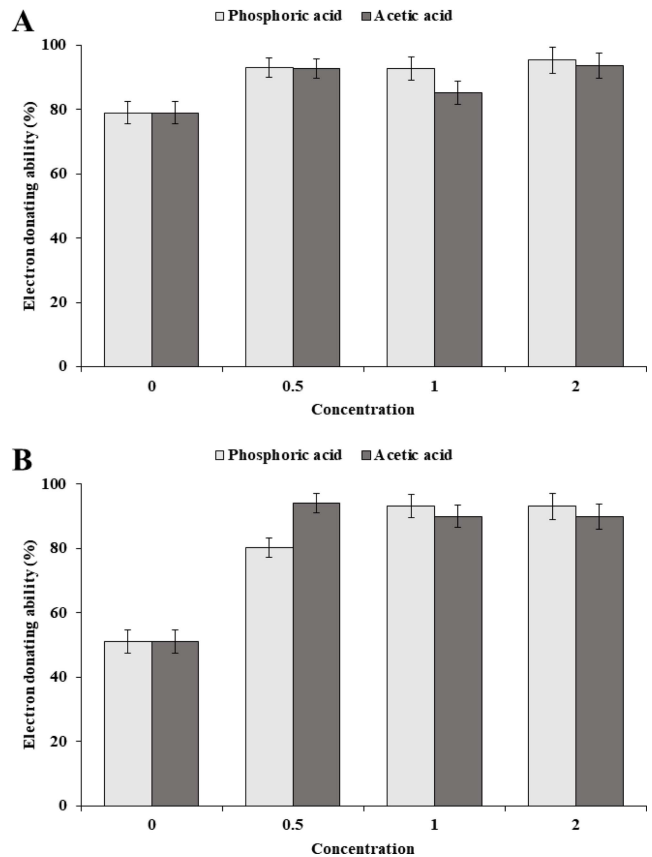


Fig. 5. DPPH free radical scavenging effect of refined Sancho oil. A; Sancho oil produced in 2012, B; Sancho oil produced in 2013.

activity of food-hazardous bacteria such as *L. monocytogenes*, *S. aureus*, and *Vibrio parahaemolyticus*. The antimicrobial activity of Sancho oil appears to be due to the essential oil component contained in a small amount in the Sancho oil. There are many reports on the antibacterial activity of plant essential oils. The essential oil components of the fruit of *Z. schinifolium* were linalool (28.2%), limonene (13.2%) and sabinene (12.1%) and showed strong antimicrobial activity against food-hazardous pathogens (Diao *et al.*, 2013).

Sancho oil, which had a high acid value, showed stronger antibacterial activity against food-hazardous bacteria than oil with low acid value. The rancidity of Sancho oil reacts with oxygen by the contact of heat, air, water, etc., and hydroperoxide is produced (Chu and Luo, 1994). In addition, it is considered that the change of unsaturated fatty acid was caused by natural oxidation of Sancho oil. Chun and Kim (1992) reported that oleic acid, linoleic acid, pentanal, and hexanal are produced when edible oils are oxidized. Yoon *et al.* (1988) reported that automatic oxidation of soybean oil reduces unsaturated fatty

acids such as linolenic acid, linoleic acid, oleic acid and increases fatty acids such as palmitic acid.

The growth of microorganisms is influenced by the content of acidic substances and the incubation temperature, and it is known that it can be controlled by controlling the pH (Lund *et al.*, 2014). Lowered acidity appears to have inhibited microbial growth. Oxidation indicates the number of carbon atoms is between 7 - 13 fatty acids with strong antibacterial action, known to represent a double bond, the material is more strong antifungal action in the these fatty acid carbon (Peck and Russ, 1947).

Sancho oil showed antifungal activity against various phytopathogens. Sancho oil has a very strong antifungal activity of *F. oxysporum*. *F. oxysporum* is a pathogenic fungus commonly found around the world. It is a soil borne ascomycete causing Fusarium wilt, on many economically important crops. This pathogen comprises of over 120 known strains and each of which is specific to unique host plant in which it causes disease (Agrios, 2005).

Assays of plant maintenance against fungi are known to be very difficult. More methods have been tried to monitor the growth rate of fungi, and the measurement of the hyphae dry weight or increased colony diameter obtained in liquid culture. In this study, the antifungal activity of Sancho oil was assayed using the agar plate-dry weight method. The agar plate-dry weight method is considered a very good method as a tool for assessing mycelial growth of vegetable oil etc.

Antimicrobial activity of phytopathogens of Sancho oil appears to be due to the fatty acids contained. Plant pathogens such as fungi, bacteria, nematodes and viruses can damage plant growth and biomass production. Harvest losses from plant diseases worldwide are known to be more than 35% in developing countries (Arici and Sanli, 2014). It occurs in the whole growing season, initially the leaves are bent and yellowed, and sometimes necrotic symptoms at the tip of the leaves progress downward. If the disease progresses severely, the whole leaf will wither and dry white. Among these pathogens, fungi and bacteria are the main pathogens and cause many diseases in plants. Most of the plants are economically reduced when they are infected with *Rhizoctonia solani*, *Pythium ultimum*, *Botrytis cinerea*, *Pythophthora capsici*, and *Erwinia caratovora* (Arici *et al.*, 2011).

Many pest control agents are very dangerous to the environment and soil, as well as methyl bromide, a pest control agent commonly used in high-value crops (Kizil *et al.*,

2005). Traditional medicine is an important source of potentially useful compounds for the development of chemotherapy agents. Various biomaterials, including plants, can be used to find effective alternatives to synthetic drugs (Gaikwadi *et al.*, 2003).

Sancho oil showed different antimicrobial activity in 6 dermatophytes. Among them, *M. canis* and *C. albicans* were the most resistant microorganisms against dermatophytes. Millions of people around the world are affected by superficial fungal infections, the most common dermatophytes. This infection, which occurs in both healthy and immunocompromised persons, is mainly caused by dermatophytes. *Trichophyton* spp. dermatophytes such as *M. canis* are generally involved in such infections (Chuang *et al.*, 2007).

They cause common infections in humans, are difficult to control effectively, and drug development is still weak. Plant-derived natural products inhibit pathogen growth without harming the host (Bokhari, 2009). Plant-derived essential oils and extracts are regarded as non-toxic compounds and have antimicrobial and anti-dermatological properties (Ibrahim and El-Salam, 2015). They have been shown to inhibit fungal pathogens (Chuang *et al.*, 2007; Ibrahim and El-Salam, 2015).

Sancho oil showed antimicrobial activity against *T. mentagrophytes* and *M. audouinii* species. This means that Sancho oil is applied to industries such as cosmetics as natural materials.

Sancho oil showed slightly more antioxidant activity after purification. Crude vegetable oils and fats contain many undesirable substances such as free fatty acids, pigments, gums, waxes, phosphates, trace metals, pesticide residues and odorous substances, which must be removed to produce a smooth, pleasant and stable product taste (Ghazani *et al.*, 2013). Most of these have an undesirable effect on the odor, odor, appearance and shelf life of the oil and must therefore be removed from the vegetable oil by a chemical or physical purification process (Verleyen *et al.*, 2002).

Sancho oil showed different antioxidant activities in different regions of production. The antioxidative activities of Sancho oil were similar or slightly lower than those of red pepper seed, pumpkin seed and camellia oil (Ku and Lee, 2018).

Antioxidant activity seems to be due to the fatty acids in the Sancho oil. The fatty acids that constitute the largest proportion of the fatty acids of Sancho oil are oleic acid, accounting for about 30% of total fatty acids, followed by linoleic acid and linolenic acid, accounting for 27% and 20%, respectively (Kim

et al., 2012). It is known to contain excellent oleic acid and palmitic acid (9% - 12%) and linoleic acid (1% - 3%), which are 85% - 90% of camellia oil and have excellent antioxidative activity (Ku and Lee, 2018).

As described above, Sancho oil showed different antimicrobial activity for each strains of food-hazardous microorganism, phytopathogenic and dermatophytes microorganism, and showed a relatively strong antimicrobial activity according to strains. While the antimicrobial activity of Sancho oil is not as strong as the solvent extracts from the leaves of the herb, it has a variety of physiological activities, which could be utilized in future cosmetics and dietary supplements.

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